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NEUROPATHIC PAIN: SOMATOSENSORY FUNCTIONS RELATED TO SPONTANEOUS ONGOING PAIN, MECHANICAL ALLODYNIA AND PAIN RELIEF

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ABSTRACT

Introduction and aim: Patients with neuropathic pain suffer from spontaneous ongoing pain and from abnormal stimulus-evoked pain, e.g., allodynia. Dynamic mechanical allodynia (DMA) is evoked by a normally innocuous light moving mechanical stimulus on the skin and static mechanical allodynia (SMA) by a sustained, normally innocuous pressure against the skin. Some patients report variable intensity of DMA which at times is only unpleasant, i.e., dynamic mechanical dysesthesia (DMD). The aim was to probe for common denominators of sensory disturbances linked to mechanisms underlying development of or protection against pain after a traumatic peripheral nerve injury (Study I). Also, we aimed at examining if short or longer lasting non-painful von Frey filament stimulation of the neuropathic skin could be used to assess perception thresholds to DMA and SMA (Study II). Further, we investigated if DMA is the hyperbole of DMD both mediated by A-beta fibres in the periphery (Study III). Finally, we explored the modulatory effect of spinal cord stimulation (SCS) on somatosensory functions within the painful area (Study IV).

Methods: Using methods of quantitative sensory testing a detailed analysis of somatosensory functions was performed in patients with and without pain after a traumatic peripheral nerve injury (Study I) and in patients reporting a sustained pain relieving effect of at least 30 % following SCS (Study IV). A compression/ischemia-induced (differential) nerve block in conjunction with repeated quantitative sensory testing of A-delta and C-fibre function was used to assess which nerve fibre population that contributes to pain at perception threshold level using 1 s (vF1) and 10 s (vF10) von Frey filament stimulation of the skin (Study II). The same approach was used to study which part of the peripheral fibre spectrum that contributes to DMA and DMD (Study III).

Results: Pain patients reported allodynia to cold and pressure in conjunction with an increase in the perception threshold to non-painful warmth on the injured side compared to the uninjured side. Pain-free patients reported hypoesthesia to light touch, cold and warmth on the injured side. No significant difference could be demonstrated comparing side-to-side differences between patients with and without pain. During the nerve block elevation of vF1 and vF10 occurred simultaneously and significantly prior to an increase in the perception level to cold or warmth. During the nerve block there was a transition of DMA to DMD in all patients with peripheral neuropathic pain and in 3/7 patients with central post stroke pain. Remaining patients lost DMA without transition. The transition/loss of DMA occurred early and concurrently in time in all patients paralleled by a continuous impairment of mainly A-beta fibre function. Following SCS decreased perception threshold to light touch and increased perception threshold to pressure pain were found in the neuropathic area when comparing with pre-stimulation values. Compared to the contralateral side these perception thresholds changed towards normalisation also including a significant normalisation of the perception threshold to non painful cold. SCS did not alter sensitivity to noxious temperature stimulation.

Conclusions: Increased pain sensitivity to cold and pressure was found on the injured side in pain patients, pointing to hyperexcitability in the pain system, not verified by a more challenging analysis of side-to side differences between patients with and without pain. A-beta fibres are the peripheral mediators of both vF1 and vF10 although different receptor organs may be involved, i.e., rapidly (RA) and slowly (SA-I) adapting mechanoreceptors, respectively. We suggest DMA to be the hyperbole of DMD, the difference being the number of mechanoreceptive fibres having access to the nociceptive system. Sensory alterations following SCS indicate a possible link to the release of a functional block on somatosensory function induced by activity in the nociceptive system. No significant correlation could be demonstrated between the degree of threshold alterations versus the degree of SCS-induced pain relief.

Keywords: Peripheral neuropathic pain; Central post stroke pain; Dynamic mechanical allodynia; Static mechanical allodynia; Dysesthesia; Quantitative sensory testing; Spinal cord stimulation.

LIST OF PUBLICATIONS

I. Landerholm ÅH, Gerber Ekblom A, Hansson PT.
Somatosensory function in patients with and without pain after traumatic peripheral nerve injury.
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II. Landerholm ÅH, Hansson PT.
The perception threshold counterpart to dynamic- and static mechanical allodynia assessed using von Frey filaments in peripheral neuropathic pain patients.
Scand J Pain, in press.

III. Landerholm ÅH, Hansson PT.
Mechanisms of dynamic mechanical allodynia and dysesthesia in patients with peripheral and central neuropathic pain.
Submitted.

IV. Landerholm ÅH, Wåhlstedt A, Hansson PT.
Somatosensory functions in patients with peripheral neuropathic pain before and after reporting pain relief from spinal cord stimulation.
Submitted.

CONTENTS

LIST OF ABBREVIATIONS

ANOVA	Analysis of variance
CNS	Central nervous system
CPSP	Central post stroke pain
CPT	Cold pain threshold
CRPS	Complex regional pain syndrome
CT	Cold perception threshold
DMA	Dynamic mechanical allodynia
DMD	Dynamic mechanical dysesthesia
HPT	Heat pain threshold
IASP	International Association for the Study of Pain
kPa	Kilo Pascal
LTT	Light touch perception threshold
mN	Milli Newton
NRS	Numerical rating scale
PNeP	Peripheral neuropathic pain
PPT	Pressure pain threshold
SCS	Spinal cord stimulation
SHP	Suprathreshold heat pain
SMA	Static mechanical allodynia
QST	Quantitative sensory testing
VAS	Visual analogue scale
WT	Warm perception threshold

1 INTRODUCTION

According to the IASP (International Association for the Study of Pain) neuropathic pain is defined as 'pain initiated or caused by a primary lesion or dysfunction in the nervous system' (Merskey and Bogduk, 1994). A new definition has recently been proposed as follows: 'pain arising as a direct consequence of a lesion or disease affecting the somatosensory system' (Treede et al., 2008). This redefinition makes it possible to more accurately differentiate neuropathic pain conditions from nociceptive pain conditions with secondary neuroplastic changes and from pain induced by spasticity or other muscular alterations due to injuries of motor nerves/central pathways.

The prevalence of neuropathic pain is not extensively studied but has been approximated to 1-8 % (Bowsher, 1991; Torrance et al., 2006). A rough estimation is that about 5 % of patients with traumatic peripheral nerve injury develop neuropathic pain (Sunderland, 1993). Central neuropathic pain has been estimated to occur in up to 8 % of patients after a stroke during a 1-year follow-up (Andersen et al., 1995). Patients suffering from neuropathic pain have reduced health-related quality of life compared to the general population (Meyer-Rosberg et al., 2001; Doth et al., 2010). Neuropathic pain is also associated with low levels of health utility and the severity of the neuropathic pain condition is a predictor of the negative health impact (Doth et al., 2010).

The diagnosis of neuropathic pain is based on a history of injury to a nervous structure or central pathway, a distribution of pain corresponding to the peripheral innervation territory of the injured nervous structure or to the topographic representation of the body part in the lesioned CNS area in conjunction with findings of sensory abnormalities within the area of pain at bedside examination (Hansson, 2002). A grading system of possible, probable and definite neuropathic pain has been proposed to determine the level of certainty with which the neuropathic pain diagnosis can be made in an individual patient (Treede et al., 2008).

1.1 TRAUMATIC PERIPHERAL NERVE INJURY

Why traumatic injuries to the peripheral nervous system result in neuropathic pain in only a fraction of inflicted patients (Sunderland, 1993) is still unknown but indicate inborn pain protective mechanisms to be the normal condition in most individuals. Besides increased peripheral activity due to, e.g., ectopic impulse discharge and ephaptic transmission, increase in spinal cord excitability has in animal models been suggested to be a built in compensation for some of the deficits in the afferent nociceptive drive after nerve injury (Chapman et al., 1998; Suzuki and Dickenson, 2000; Suzuki et al., 2000). Also, disinhibition of spinal neurones due to loss of peripheral input may come into play (Castro-Lopes et al., 1993; Moore et al., 2002). A mechanism-based classification of neuropathic pain is not available and it is currently not possible to translate clinical symptoms and signs into identified distinct pathophysiological mechanisms thereby linking therapy to pain mechanisms (Hansson, 2003; Baron et al., 2010). To eventually accomplish this there is a need for a continuous collaboration among clinicians and basic scientist.

Patients with neuropathic pain, spontaneous and/or abnormal stimulus-evoked pain, of peripheral traumatic origin may present with seemingly random combinations of both qualitative and quantitative sensory abnormalities in the innervation territory of the injured nervous structure (Lindblom and Tegner, 1985; Hansson and Kinnman, 1996; Pertovaara, 1998). No pathognomonic somatosensory aberration patterns have so far been identified in such patients. Since a mechanism-based classification is not available searching for common denominators of sensory disturbances may provide links to mechanisms underlying pain development and maintenance after traumatic peripheral nerve injury. Meticulous somatosensory testing with the aim of phenotyping subgroups of patients with and without pain based on somatosensory findings and symptoms could be a first step towards such a link. Sensory disturbances with bearing on development of spontaneous pain after nerve injury could reasonably be expected to be related to alterations in activity in nociceptive channels. Previous attempts comparing sensory findings from patients with traumatic peripheral nerve injuries with and without pain are scarce (Gottrup et al., 2000; Jaaskelainen et al., 2005; Aasvang et al., 2008). The findings of allodynia to mechanical pressure and abnormal temporal summation of pinprick pain on the affected side compared to the normal side were interpreted by the authors as signs of peripheral and/or central hyperexcitability contributing to spontaneous pain while hypoesthesia to non-nociceptive stimuli could not define patients with and without pain (Gottrup et al., 2000; Jaaskelainen et al., 2005; Aasvang et al., 2008).

1.2 CENTRAL POST STROKE PAIN

Central post stroke pain (CPSP) is less studied than peripheral neuropathic pain conditions and is a challenge to the clinician when it comes to diagnosis and treatment. Patients with CPSP complain of continuous ongoing pain located in the area corresponding to the topographic representation of the body part in the lesioned CNS area and sometimes also from stimulus evoked pain, e.g., allodynia. Patients with CPSP all have a lesion that affects temperature- and pain sensibility, i.e., the spino-trigemino-thalamo-cortical pathway (Leijon et al., 1989; Vestergaard et al., 1995) however all patients with a lesion to the spino-trigemino-thalamo-cortical do not develop CPSP. Only a minority of patients have a lesion affecting vibration and tactile sensibility (Boivie et al., 1989). Disinhibition following deafferentation, sensitization and plasticity changes, all hypothetical phenomena, have been suggested to underlie the development of CPSP (Craig, 2007).

1.3 MECHANICAL ALLODYNIA AND DYSESTHESIA

Patients with neuropathic pain usually present with spontaneous ongoing pain and sometimes with additional abnormal stimulus-evoked pain (e.g., allodynia) (Cruccu et al., 2004). Not infrequently also abnormal spontaneous and/or evoked non-painful sensory phenomena such as paresthesia and dysesthesia are reported. According to the IASP allodynia is defined as ‘pain due to a stimulus which does not normally provoke pain’ and dysesthesia is defined as ‘an unpleasant abnormal sensation, whether spontaneous or evoked’ (Merskey and Bogduk, 1994). Two types of mechanical allodynia have been distinguished in studies of neuropathic pain patients; dynamic mechanical allodynia (DMA) and static mechanical allodynia (SMA) (Koltzenburg et al., 1992; Ochoa and Yarnitsky, 1993). Clinically, DMA is evoked by a normally

innocuous light moving mechanical stimulus on the skin and SMA by a light sustained normally innocuous pressure against the skin.

DMA is an oppressive symptom in subgroups of patients with neuropathic pain interfering extensively with activity of daily living (Smith and Sang, 2002; Hensing et al., 2007). The prevalence of DMA has been reported en passant in limited groups of patients in studies aiming at other issues (Leijon et al., 1989; Andersen et al., 1995; Gottrup et al., 2000; Martin et al., 2003; Otto et al., 2003; Greenspan et al., 2004) and seems to be present in a minority of patients. In a recent large study of patients with neuropathic pain the prevalence of DMA was reported to be 20 % across diagnostic entities, most frequently found in patients with post herpetic neuralgia (49%) (Maier et al., 2010). The high prevalence in this patient group could possibly be explained by the presence of peripheral sensitization/neurogenic inflammation in subgroups of patients.

Clinical empiricism supports the notion of similar perceptual characteristics reported by patients with DMA of peripheral and central neuropathic origin despite completely different lesion levels. It has been suggested that DMA usually has a distribution within the entire or part of the proper innervation territory of the lesioned peripheral nervous structure or central pathway and in most patients is a constant finding (Hansson, 2003). However, in subgroups of patients it is a clinical observation that the phenomenon varies in intensity and at times only an unpleasant, i.e., dysesthetic sensation, or even just a sensation of touch can be evoked by lightly touching the skin. Dynamic mechanical dysesthesia (DMD) and DMA may also coexist in different areas of the neuropathy distribution and in the clinical setting most patients are usually able to differentiate between a painful and a dysesthetic sensation during examination.

In peripheral neuropathic pain conditions there are several lines of evidence in the literature indicating different peripheral nerve fibre correlate to DMA and SMA, respectively. With the exception of, e.g., subgroups of patients with post herpetic neuralgia and nociceptor sensitisation (Fields et al., 1998) DMA is claimed to be mediated by myelinated fibres (Koltzenburg et al., 1992; Ochoa and Yarnitsky, 1993; Kilo et al., 1994; Field et al., 1999) and SMA by C-fibres (Koltzenburg et al., 1992; Ochoa and Yarnitsky, 1993; Kilo et al., 1994; Field et al., 1999) but also A-delta fibres have been implicated in the static subtype (Field et al., 1999; Ziegler et al., 1999). Other afferents than low threshold A-beta mechanoreceptive fibres may, however also be suggested as possible candidates mediating DMA in patients with PNeP. Experimental animal studies have identified nociceptive A-beta fibres (Cain et al., 2001; Djouhri and Lawson, 2004) and in humans A-delta low-threshold mechanoreceptors have been reported (Adriaensen et al., 1983). In primates C-fibre nociceptors with low mechanical threshold have been documented (Slugg et al., 2000) and in human skin low-threshold mechanoreceptive C-fibres involved in touch sensation (Vallbo et al., 1993; McGlone et al., 2007). Peripheral sensitization (Fields et al., 1998), ephaptic transmission between A-beta fibres and nociceptive fibres (Amir and Devor, 1992), alterations in spinal cord excitability (Laird and Bennett, 1992), central sensitization (Fields et al., 1998), descending facilitation of dorsal horn neurons (Suzuki et al., 2002) and sprouting of mechanoreceptive fibers from deeper- to more superficial layers of the dorsal horn where synaptic couplings to nociceptive neurons may take place (Woolf et al., 1992; Woolf et al., 1995; Bao et al., 2002) have also been

suggested as possible pathophysiological mechanisms underlying DMA. The pathophysiological basis for DMA in patients with central post-stroke pain (CPSP) is unknown. The neurophysiological basis for DMD has, to our knowledge, never been addressed.

Non-quantitative brushing stimuli are usually employed to induce DMA (Leijon et al., 1989; Andersen et al., 1995; Gottrup et al., 2000; Martin et al., 2003; Otto et al., 2003; Greenspan et al., 2004) and moving a brush across the non-inflamed skin surface is likely to result in dynamic activation and deactivation of both rapidly adapting (RA) and slowly adapting (SA) mechanoreceptors (Johnson, 2001; Lundstrom, 2002; Samuelsson et al., 2005). A brief vertical, about 1 second (s), stimulation of the skin with a thin von Frey filament causes a dynamic deformation of the skin surface and an "on-off" activation of A-beta mechanoreceptors (Johansson et al., 1980). Hence, a vertical deformation of the skin induced by stimulation with a von Frey filament activates the same peripheral substrate as a horizontally moving brush although the spatial activation pattern is lacking using the former.

Various types of mechanical stimuli have been used to study hypersensitivity to static mechanical stimuli, some activating non-nociceptive- and some nociceptive fibres; von Frey filament prodding (Attal et al., 1999; Wallace et al., 2002), gentle manual pressure or pinching (Ochoa and Yarnitsky, 1993), pressure-algometry (Koltzenburg et al., 1992) and tonic pressure (Kilo et al., 1994). Mechanical stimulation by prodding the skin with a von Frey filament is likely to result in activation of mechanoreceptors and also a possibility of activating nociceptors, sensitized or not, the latter not necessarily resulting in pain perception (Adriaensen et al., 1983; Schmidt et al., 1995; Andrew and Greenspan, 1999; Slugg et al., 2000). In addition, the duration of the "static" stimulus varied and no gold standard has been defined. Under normal conditions a light sustained non-painful pressure applied to the skin results in activation of A-beta mechanoreceptors (Guyton and Hall, 2000). In patients with peripheral neuropathic pain and SMA, based on the aforementioned published data, a sustained, about 10 s indentation of the skin with a von Frey filament would hypothetically result in activation of epidermal C- and A-delta nociceptors (Garell et al., 1996; Treede et al., 2002).

1.4 SPINAL CORD STIMULATION

The treatment options for neuropathic pain conditions are limited and often provide only partial pain relief. Besides pharmacotherapy (Attal et al., 2010) one of the treatment options in peripheral neuropathic pain conditions is spinal cord stimulation (SCS) which evolved as a direct consequence from the gate-control theory (Melzack and Wall, 1965). However, the pathophysiological mechanisms causing pain relief in peripheral neuropathic pain conditions are to a large extent unknown despite four decades of experience using SCS. In contradiction to the original theory the method is not efficacious in acute nociceptive pain conditions but has been proven to be so in patients with painful radiculopathy in failed back surgery syndrome (FBSS) (Cruccu et al., 2007; Kumar et al., 2007). The method is suggested to antidromically activate the dorsal columns mediating inhibitory activity into the dorsal horn (Meyerson and Linderoth, 2006) and/or via supraspinal loops including descending inhibition thus

reducing spinal hyperexcitability (Saade et al., 2006; Song et al., 2009). In experimental animal models of neuropathy, not necessarily painful, SCS has been demonstrated to inhibit hyperexcitability in the dorsal horn by inducing release of numerous neurotransmitters, e.g. GABA (Cui et al., 1997a), adenosine (Cui et al., 1997b; Cui et al., 1998), serotonin and noradrenalin (Linderoth et al., 1992; Linderoth and Foreman, 2006) and acetylcholine (Schechtmann et al., 2004; Schechtmann et al., 2008) possibly restoring the balance between excitation/inhibition in the dorsal horn.

Peripheral neuropathic pain patients present with seemingly random profiles of sensory abnormalities in the innervation territory of the injured nervous structure (Hansson and Kinnman, 1996) reflecting assessed loss of and (over-) compensated function, i.e., hypoesthesia and allodynia/hyperalgesia. Loss may result from deafferentation and both loss and compensated function has been demonstrated to result from activity in the nociceptive system affecting somatosensory function in nociceptive and non-nociceptive channels (Leffler et al., 2000; Geber et al., 2008) which originally was suggested by others (Loh and Nathan, 1978; Lindblom and Verrillo, 1979). The latter, a functional block might explain improved sensitivity reported after pain relief in patients with neuropathy (Lindblom and Verrillo, 1979; Marchettini et al., 1992). Only few previous studies on very limited groups of patients have investigated the correlation between the modulatory effect of SCS on somatosensory function within the painful area and the relief of spontaneous pain in patients with painful neuropathy (Lindblom and Meyerson, 1975; Lindblom and Meyerson, 1976; Marchand et al., 1991; Eisenberg et al., 2006). The results are inconsistent possibly due to presence of nociceptive pain with referred pain components in part of the studied patient groups influencing somatosensory perception (Leffler et al., 2000).

In this thesis, using methods of quantitative sensory testing a detailed analysis of somatosensory functions related to spontaneous ongoing pain, mechanical allodynia and pain relief was performed in patients with neuropathic pain. The purpose was to probe for common denominators of sensory disturbances linked to mechanisms underlying development of or protection against pain following a traumatic peripheral nerve injury. In addition, the aim was to examine if short or longer lasting non-painful von Frey filament stimulation of the neuropathic skin could be used to assess perception thresholds to dynamic mechanical and static mechanical allodynia. This could be used in intervention studies aimed at modifying such stimulus-evoked phenomena bearing in mind that suprathreshold stimuli is the every day problem in patients suffering from mechanical allodynia. Further, we aimed at examining if dynamic mechanical allodynia in patients with neuropathic pain could be the hyperbole of dynamic mechanical dysesthesia both mediated by A-beta fibres in the periphery. Finally, the thesis work probed the modulatory effect of spinal cord stimulation on somatosensory functions within the painful area because such an approach may disclose details about the link between sensory aberrations and spontaneous ongoing pain.

2 AIMS OF THE THESIS

2.1 SPECIFIC AIMS

2.1.1 Study I

To examine the function of somatosensory systems in patients with traumatic peripheral nerve injury with and without pain probing for common denominators of sensory disturbances that may provide possible links to mechanisms underlying development of or protection against pain. Further, we aimed at extending the results of others by challenging the pain system using magnitude estimation of suprathreshold heat pain stimuli in patients with possibly less confounding trauma-related factors than in previous studies.

2.1.2 Study II

To examine if short (1 s) or longer (10 s) lasting usually non-painful von Frey filament stimulation of the neuropathic skin in patients with painful traumatic peripheral nerve injury could be used to assess perception thresholds to dynamic mechanical allodynia and static mechanical allodynia. Techniques to quantify the different allodynias at perception threshold level are in demand as adjuncts to suprathreshold stimuli in intervention studies aimed at modifying these stimulus-evoked phenomena.

2.1.3 Study III

To examine if dynamic mechanical allodynia in patients with peripheral neuropathic pain or central post stroke pain could be the hyperbole of dynamic mechanical dysesthesia both mediated by A-beta fibres in the periphery. We hypothesised that allodynia would transfer to a dysesthetic sensation during the successive compression-ischemia blocking of A-beta fibres.

2.1.4 Study IV

To investigate the modulatory effect of spinal cord stimulation on somatosensory functions within the painful area in a larger group of patients with neuropathic pain following an injury to a peripheral nerve or nerve root without concomitant pain of non-neuropathic origin. Furthermore, we aimed at extending observations of others by including an analysis of the correlation between changes in somatosensory functions and the degree of pain relief following spinal cord stimulation. Such comparisons may disclose details about the link between sensory aberrations and spontaneous ongoing pain.

3 MATERIAL AND METHODS

3.1 SUBJECTS

All participating patients with neuropathic pain were out patients recruited from Pain Center, Department of Neurosurgery, Karolinska University Hospital, Solna or from Multidisciplinary Pain Center, Uppsala University Hospital. Patients without pain (Study I) were recruited from Department of Hand Surgery, Södersjukhuset, Stockholm. All patients were diagnosed by a neurologist (author P.H. or Å.L.). A partial peripheral nerve injury was defined as remaining sensibility of any modality in part of or in the entire innervation territory of the injured nervous structure and no history or chart notes of total anaesthesia in the same territory, indicating complete nerve lesion, at the time of injury.

Exclusion criteria in patients with peripheral nerve injuries were complete nerve lesions, bilateral lesions of peripheral nerves, clinical signs of overt neurogenic inflammation or autonomic dysfunction, a diagnosis of CRPS type II, age <18 or >80 years, pain of non-neuropathic origin in the pain affected or contralateral area, systemic diseases predisposing for neuropathy or severe somatic or psychiatric diseases. If the patient was treated with a spinal cord stimulator this had to be turned off for at least 12 hours before examination to allow for the pain relieving effect to cease. All patients volunteered no remaining post stimulatory pain relief before start of the test session. Ongoing pharmacological treatment of the painful condition was allowed. All studies were performed in accordance with the Declaration of Helsinki and were approved by the local ethical committee of the Karolinska University Hospital, Solna and all subjects gave their informed consent to participation.

3.1.1 Study I

Thirty-four patients with unilateral partial peripheral traumatic nerve injury were studied.

3.1.1.1 Patients with pain

Eighteen patients with spontaneous ongoing pain (12 females, 6 males, median age 46 years, range 25 – 59 years) participated. The median duration of time since nerve injury was 6 years (range 1 – 15 years). Inclusion criteria for patients with pain were a duration of pain >6 months and pain intensity immediately preceding the study examination of at least 30/100 on a 0 – 100 points numerical rating scale (NRS) (0 = no pain, 100 = worst pain imaginable). The nerve injury had not been surgically sutured in any of the patients reporting pain. Demographic data is shown in Table 1.

Table 1.
Demographic data of patients with painful unilateral partial peripheral traumatic nerve injury (n=18).

Patient (gender)	Age (years)	Pain duration (years)	Injured nerve	Etiology of nerve injury	Spontaneous ongoing pain (NRS)	Medication	SCS
1 M	29	10	Saphenous nerve	Compartment syndrome, lower leg including fasciotomy	30/100	None	No
2 F	59	3	Saphenous nerve	Knee joint replacement surgery	39/100	AMI	No
3 F	51	3	Radial nerve	Surgery of proximal humerus fracture	80/100	AMI, GAB	No
4 M	25	1	Intercostal nerves T5-9	Thoracotomy due to pneumothorax	40/100	None	No
5 F	48	2	Iliohypogastric /ilioinguinal nerves	Abdominal surgery	70/100	None	No
6 F	49	2	Saphenous nerve	Compartment syndrome, lower leg including fasciotomy	35/100	None	No
7 F	32	8	Lateral cutaneous nerve of the thigh	Laparoscopic abdominal surgery	75/100	None	No
8 F	59	9	Anterior cutaneous nerves of the thigh	Surgery due to trochanter bursitis	50/100	None	No
9 M	47	11	Tibial nerve	Stab injury and fasciotomy, lower leg	50/100	None	No
10 M	29	3	Anterior cutaneous nerves of the thigh	Surgery/stripping of varicous veins	40/100	None	No
11 F	36	15	Sural nerve	Ankle joint surgery	60/100	None	No
12 F	39	3	Superficial radial nerve	Thenotomy at wrist level	45/100	None	No
13 M	46	9	Tibial nerve	Gun shot injury in the lower leg	50/100	None	No
14 F	45	10	Tibial nerve	Surgery due to local infection/necrosis in the calcaneus	30/100	None	No
15 F	27	4	Median nerve	Carpal tunnel surgery	60/100	PAR, COD	No
16 F	37	9	Median nerve	Compression due to humerus fracture	45/100	None	Yes
17 M	58	6	Ulnar nerve	Elbow joint surgery	70/100	None	Yes
18 F	51	5	Ulnar nerve	Surgery at elbow level due to lipoma	100/100	None	Yes

M, male; F, female; NRS, numerical rating scale; AMI, amitriptyline; COD, codeine; GAB, gabapentin; PAR, paracetamol.

3.1.1.2 Patients without pain

Sixteen patients presented without pain (4 females, 12 males, median age 31 years, range 19 – 62 years). Median duration of time since nerve injury was 4 years (range 1 – 7 years). Inclusion criteria for patients without pain were duration of > 6 months since the nerve injury and a subjective experience of sensory abnormality to at least one modality in the innervation territory of the injured nerve. In patients without pain there was information in the patients' charts about the degree of nerve injury based on visual inspection made by the surgeon during surgery. Only partial nerve injuries were included. The nerve injury had been surgically sutured in all patients. Demographic data is shown in Table 2.

Table 2.
Demographic data of patients with unilateral partial peripheral traumatic nerve injury without pain (n=16). All patients had cut injuries at wrist level or distally in the hand.

Patient (gender)	Age (years)	Time since injury (years)	Injured nerve
19 M	62	6	median nerve
20 F	32	6	median nerve
21 M	62	5	median nerve
22 F	24	3	median nerve
23 F	23	4	median nerve
24 M	23	2	median nerve
25 M	47	6	median nerve
26 M	23	7	median nerve
27 M	25	6	ulnar nerve
28 M	29	1	ulnar nerve
29 M	19	4	median nerve
30 M	45	4	median nerve
31 M	43	3	median nerve
32 F	23	1	median nerve
33 M	32	2	ulnar nerve
34 M	35	4	ulnar nerve

M, male; F, female

3.1.2 Study II

Eighteen patients with painful unilateral partial peripheral traumatic nerve injury in a limb were studied (12 females, 6 males, median age 51 years, range 24 – 62 years). The median duration of time since nerve injury was 5 years (range 1 – 12 years). All patients presented with DMA and 9 patients reported concomitant SMA in the area of DMA. DMA was considered to be present if pain was evoked by stroking the neuropathic skin with a camel's hair brush, and SMA if sustained prodding perpendicularly against the skin for 10 s using a q-tip was painful for the duration of the stimulation. No patient had any previous experience with a compression/ischemia-induced (differential) nerve block. Inclusion criteria were duration of pain >6 months and only patients with DMA with or without concomitant SMA were eligible. These

patients also participated in another study on mechanical allodynia (Study III) and were examined with additional quantitative sensory testing during the same session. Demographic data is shown in Table 3.

Table 3.
Demographic data of patients with painful unilateral partial peripheral traumatic nerve injury in a limb (n=18).

Patient (gender)	Age (years)	Pain duration (years)	Injured nerve	Spontaneous ongoing pain (VAS)	SMA	SCS	Medication
1 M	28	7	Lateral cutaneous nerve of the forearm	10/100	No	Yes	None
2 F	60	3	Ulnar nerve	0/100	No	No	None
3 F	53	2	Brachial plexus	0/100	Yes	Yes	None
4 M	30	11	Sural nerve	50/100	No	No	None
5 F	24	2	Median nerve	42/100	No	No	None
6 F	38	4	Lateral cutaneous nerve of the calf	30/100	Yes	No	None
7 F	51	3	Ulnar nerve	32/100	Yes	No	None
8 M	39	5	Digital nerve of finger	72/100	Yes	Yes	None
9 F	51	12	Medial cutaneous nerve of the forearm	80/100	Yes	No	PAR
10 F	62	6	Ulnar nerve	60/100	No	No	PRE
11 M	43	5	Digital nerve of finger	70/100	Yes	No	None
12 F	56	6	Infra patellar nerve	20/100	Yes	No	PAR, COD
13 F	52	8	C8 root	55/100	Yes	No	PRE, DUL
14 F	46	7	L5 root	85/100	Yes	No	None
15 F	50	1	Superficial peroneal nerve	65/100	No	No	TRA, DUL
16 M	45	8	Superficial peroneal nerve	35/100	No	No	None
17 M	54	5	Ulnar nerve	30/100	No	Yes	None
18 F	58	3	Median nerve	40/100	No	No	CEL

SMA, static mechanical allodynia; M, male; F, female; VAS, visual analogue scale. Intensity of spontaneous ongoing pain translated from a 100 mm visual analogue scale (VAS), graded from 0 = 'no pain at all' to 100 = 'worst possible pain', as rated during the clinical examination immediately preceding the experiment.
SCS, spinal cord stimulation; CEL, celecoxib; COD, codeine; DUL, duloxetine; PAR, paracetamol; PRE, pregabalin; TRA, tramadol;.

3.1.3 Study III

Twenty five patients with neuropathic pain and DMA in a limb were included. Inclusion criteria were duration of pain >6 months. DMA was considered to be present if pain was evoked by stroking the skin with a camel's hair brush. Before the experiment none of the patients presented with dysesthesia only in the area examined with the brush.

3.1.3.1 Patients with peripheral neuropathic pain

Eighteen patients with peripheral neuropathic pain (PNeP) due to a unilateral partial peripheral traumatic nerve injury in a limb were studied (12 females, 6 males, median age 51 years, range 24-62 years). The median duration of time since nerve injury was 5 years (range 1-12 years).
None of the patients with PNeP pain had undergone neurophysiological examinations. These patients also participated in another study on mechanical allodynia (Study II) and were examined with additional quantitative sensory testing during the same session. Demographic data is shown in Table 3.

3.1.3.2 Patients with central post stroke pain

Seven patients with central post stroke pain (CPSP) were studied (3 females, 4 males, median age 68 years, range 38 – 70 years). The median time of disease duration was 3 years (range 0.25 – 11 years). All patients had their cerebral lesion verified by computerized tomography (CT).
Additional exclusion criteria in this patient group were a clinical history of bilateral cerebrovascular lesions or bilateral lesions visible on CT, signs of cognitive dysfunction or neglect, marked paralysis of the painful hand and a history of injury to the peripheral nervous system in the contralateral non-painful limb.
Demographic data is shown in Table 4.

Table 4.
Demographic data on patients with central post stroke pain (n=7).

Patient (gender)	Age (years)	Pain duration (years)	Location of cerebral lesion (computerized tomography)	Location of examination area with DMA	Spontaneous ongoing pain (VAS)	Medication
19 M	69	11	left dorsal putamen/posterior internal capsule hemorrhage	right hand	82/100	GAB TRA PAR
20 F	42	2	right putamen hemorrhage	left hand	66/100	GAB
21 M	60	3	left posterior internal capsule hemorrhage/infarct	right hand	81/100	AMI GAB PAR
22 F	70	2	right dorsolateral thalamus/posterior internal capsule hemorrhage	left hand	43/100	AMI
23 M	66	8	left dorsal basal ganglia/posterior internal capsule hemorrhage	right hand	40/100	TRA
24 F	68	11	left caudate nucleus, posterior internal capsule and lateral thalamic hemorrhage	right hand	100/100	AMI GAB
25 M	38	0.25	right dorsal putamen hemorrhage	left foot	50/100	PRE

VAS, visual analogue scale; Intensity of spontaneous ongoing pain translated from a 100 mm visual analogue scale (VAS), graded from ‘no pain at all’ to ‘worst possible pain’, as rated during the clinical examination immediately preceding the experiment. AMI, amitriptyline; GAB, gabapentin; PAR, paracetamol; PRE, pregabalin; TRA, tramadol.

3.1.4 Study IV

Sixteen patients with painful unilateral partial traumatic injury to a peripheral nerve or nerve root reporting pain relief from spinal cord stimulation (SCS) were studied (11 females, 5 males, median age 48 years, range 37-64 years). The median time since nerve injury was 9 years (range 3 – 15 years) and median time since implant of the SCS-system was 2.5 years (range 3 months – 9 years). Inclusion criteria were a duration of spontaneous ongoing pain >6 months, time since implant of the spinal cord stimulator of at least 3 months and only patients reporting a sustained post stimulatory pain relieving effect of at least 30 % lasting no less than 45 minutes were eligible. The median pain relieving effect induced by SCS was 68 % (range 43 – 100%) and the median stimulation time to induce the usual magnitude of pain relief was 39 min (range 30 – 60 min). Following SCS no patient reported any fading of the pain relieving effect during the post stimulatory examination and all rated their pain intensity identical before and after the examination. Only 5/16 patients participating had undergone magnetic resonance tomography and 1/16 patients neurophysiological examination confirming the diagnosis of injury to a nervous structure. Therefore, the diagnosis of neuropathic pain can merely be assessed with the highest degree of certainty, i.e., ‘definite’ neuropathic pain, in 6/16 patients according to the recently proposed grading system (Treede et al., 2008). Hence, we only claim that ‘probable’ neuropathic pain was at hand in 10/16 patients. Demographic data is shown in Table 5.

Table 5.
Demographic data on patients with painful unilateral partial traumatic injury to a peripheral nerve or nerve root reporting pain relief from spinal cord stimulation (n=16).

Patient (gender)	Age (years)	Pain duration (years)	Injured nervous structure	Duration since implant of SCS (years /months)	Spontaneous ongoing pain before SCS (NRS)	Spontaneous ongoing pain after SCS (NRS)	Stimulation time (min)	Medication
1 F	63	5	left S2 root	2	70/100	30/100	40	None
2 M	47	11	right S1 root	7	50/100	20/100	30	AMI, PAR, TRA
3 F	37	9	right median nerve	7	45/100	5/100	40	None
4 F	64	3	left L5 root	6 months	50/100	25/100	40	TRA
5 F	56	5	left brachial plexus	4	70/100	40/100	40	COD, PAR
6 F	44	11	left L5 root	9	30/100	0/100	40	None
7 M	48	4	left ulnar nerve	3 months	70/100	40/100	50	None
8 F	38	10	right S1 root	9 months	40/100	20/100	60	None
9 F	51	7	right ulnar nerve	10 months	100/100	10/100	35	None
10 M	60	9	right L5 root	7	40/100	10/100	32	PAR
11 F	46	9	right intercostal nerves T8-T10	5	70/100	10/100	40	None
12 F	41	6	left S1 root	3	80/100	45/100	30	None
13 M	50	10	right L5 root	3 months	50/100	10/100	35	None
14 M	56	4	right L5 root	2	50/100	0/100	37	None
15 F	47	11	right L5 root	8	70/100	40/100	32	None
16 F	44	15	left S1 root	1	30/100	5/100	30	GAB

M, male; F, female; NRS, numerical rating scale; SCS, spinal cord stimulation; AMI, amitriptyline; COD, codeine; GAB, gabapentin; PAR, paracetamol; TRA, tramadol.

3.2 METHODS

3.2.1 General procedure

The diagnosis of neuropathic pain was based on a history of injury to a peripheral nerve or nerve root or a cerebrovascular lesion, a distribution of pain corresponding to the peripheral innervation territory of the injured nervous structure or to the topographic representation of the body part in the lesioned CNS area in conjunction with findings of sensory abnormalities within the area of pain at bedside examination. To guide sensibility testing patients were asked to indicate the area of spontaneous ongoing pain, DMA and SMA (if present) and in patients without pain the area of subjective sensory disturbance on a body drawing. Before the test session patients were asked to rate the intensity of spontaneous ongoing pain on a 100 mm visual analogue scale (VAS) (Study II and III) or on a 0 – 100 points numerical rating scale (NRS) (0 = no pain, 100 = worst pain imaginable) (Study I and IV). All patients underwent a neurological examination by author Å.L. including a bedside examination of the somatosensory systems (touch, warmth, cold and pin prick) as part of the diagnostic work-up. Somatosensory functions were also monitored by quantitative sensory testing (QST), see below. All tests and sensibility assessments were performed by author Å.L. in a quiet room with the patient comfortably seated in a chair or lying in a relaxed supine position on a bed. Before the test session patients were carefully familiarized with the different methods to be used and to the testing procedure. The patients were instructed to keep their eyes closed during the tests and were unaware of the test results during the session. To assure that the subsequent assessments all were made in the same location an examination point/area was marked with a pen. Care was taken to choose an examination point/area within the area of sensory aberration or maximum pain, DMA or SMA where the examination device was possible to apply and to stimulate the same point/area for all types of stimuli. All sensibility testing was done first in the corresponding contralateral non-injured area and then in the projection area of the injured nervous structure or in the topographic representation of the body part in the lesioned CNS area. The method of limits was used in all quantitative testing of somatosensory perception thresholds except in testing the perception threshold to light touch using von Frey filaments where the method of levels was used.

3.2.1.1 Study I

Perception thresholds to warmth, cold, light touch, pressure pain, cold- and heat pain were assessed as were pain intensities at suprathreshold heat pain stimulation. In patients with pain the testing was made within the area of maximum pain and in patients without pain in the area with sensory disturbance.

3.2.1.2 Study II and III

Baseline sensibility testing, i.e., perception thresholds to light touch, cold, warmth, cold- and heat pain was made to get an estimation of the degree of sensory dysfunction in the area of DMA/SMA. In the neuropathic area also pain perception thresholds to von Frey filament stimulation of 1 s and 10 s were assessed. Finally, a control area with no signs of neuropathy was examined to obtain baseline values for cold and warm perception thresholds subsequently used to monitor the progression of a

compression/ischemia-induced (differential) nerve block of the painful limb. In patients with PNeP the control area was located neighbouring the neuropathic area at the same proximo-distal level but in patients with CPSP the control area was located in the corresponding homologous non-painful area.

3.2.1.3 Study IV

In all patients perception thresholds to warmth, cold, light touch, pressure pain, cold- and heat pain were assessed as were pain intensities at suprathreshold heat pain stimulation. Half of the patients were examined with mechanical stimulation preceding thermal stimulation and the other half in the reverse order. The patients were then instructed to turn on the spinal cord stimulator and stimulate with their regular duration of stimulation until their usual level of pain relief was obtained. Stimulation time varied between 30-60 minutes. The spinal cord stimulator was then turned off and patients were re-examined in the neuropathic area. Both immediately before and after the post stimulatory examination patients were asked to rate the intensity of spontaneous ongoing pain. The post stimulatory testing protocol lasted approximately 20 min.

3.2.2 Quantitative sensory testing

3.2.2.1 Perception threshold to light touch

Perception threshold to light touch was assessed using a set of 15 von Frey filaments (OptiHair®, Marstock-nervtest, graded from 0.29 mN (0,03 gram) to 294 mN (30 gram) (logarithmical increase) (Fruhstorfer et al., 2001) made of optical glass fibre. To keep the contact surface approximately constant for various fibre diameters the tip of the fibre is coated with a tiny round epoxy bead (diameter about 0.5 mm). This may also reduce the risk of nociceptor activation compared to conventional nylon monofilaments with sharp edges (Greenspan and McGillis, 1991; Magerl et al., 1998). Care was taken to apply the filaments perpendicularly to the surface of the skin and avoiding contact with body hair, shaving the skin if necessary. The light touch perception threshold (LTT) in each area was calculated as the mean value of five descending and five ascending assessments (Kosek et al., 1996).

3.2.2.2 Pain perception thresholds to von Frey filament stimulation (Study II)

Pain perception thresholds to mechanical stimulation were assessed using the same set of von Frey filaments as the LTT (see above). We used a stimulus duration of approximately 1 s or 10 s aiming at assessing the perception threshold counterpart to DMA and SMA. The baseline von Frey pain perception thresholds were defined as the lowest pressure considered painful for the different stimulus duration of 1 s (vF1) and 10 s (vF10), respectively. All patients were examined with both stimulus durations, the short stimulus always preceding the longer one. The average of three ascending perception levels was calculated as the baseline von Frey pain perception threshold.
Importantly, the von Frey filaments used in the study did not evoke pain in the contralateral pain free area or in the control area neighbouring the neuropathic skin in any patient.

3.2.2.3 Pressure pain threshold (Study I and IV)

The pressure pain threshold (PPT) was assessed using a pressure algometer (Somedic Sales AB, Hörby, Sweden). A circular padded probe with an area of 1 cm² was used and the perception level to pressure pain was assessed three times, with an average pressure application rate of 50 kPa/s and an interstimulus interval of 15 seconds. The patients were instructed to press a hand-held button as soon as the pressure turned into a painful sensation, whereby the pressure value was frozen on a digital display. The mean value of the last two perception levels was calculated as the PPT.

3.2.2.4 Thermal perception thresholds

Thermal thresholds were assessed using a Peltier element based thermode of 12.5 cm² (Modular Sensory Analyser, Somedic Sales AB, Hörby, Sweden) applied to the skin. If necessary the thermode was secured to the skin with an elastic bandage to keep it in place, care taken to apply minimal pressure. The baseline temperature of the thermode was set equal to the skin temperature assessed with the infrared skin temperature analyzer Tempett® (Somedic Sales AB, Hörby, Sweden) and then adjusted manually until the patient perceived the sensation of the thermode as indifferent. The perception thresholds to non-painful cold (CT) and warmth (WT) were obtained by delivering five cold followed by five warm stimuli with a preset randomised inter-stimulus interval of 4-10 s and with a stimulus rate of 1ºC/s. The patients were instructed to press a hand-held button at the first sensation of cold or warmth, respectively, thereby terminating the stimulus. Thresholds were calculated as the average temperature difference from skin temperature (baseline) of five successive perception levels (ΔCT, ΔWT).
The noxious temperatures were delivered manually. The patients were instructed to press a hand-held button at the first sensation of pain thereby terminating the stimulus. The perception thresholds to heat- (HPT) and cold pain (CPT) were calculated as the mean value of three (Study I) or as the mean value of the last two of three (Study IV) successive perception levels with a stimulus rate of 1ºC/s and an inter-stimulus interval of 30 seconds. To avoid tissue damage the maximum temperature was set at 50ºC and the minimum temperature at 5 (Study II and III) or 10ºC (Study I and IV), respectively. Failure to respond before the cut-off limit was reached resulted in assignment of the cut-off value.

3.2.2.5 Suprathreshold heat pain stimulation (Study I and IV)

The sensitivity to suprathreshold heat pain (SHP) was assessed with a stimulus rate of 1ºC/s and an inter-stimulus interval of 3 minutes. To avoid tissue damage the maximum temperature was set at 50ºC. The patients were instructed to push the button immediately when they would rate the heat pain intensity as 60 out of 100 on a NRS (SHP 60/100). SHP 60/100 was calculated as the mean value of two successive measurements. To be able to create stimulus-response functions for suprathreshold heat pain the interval between HPT and SHP 60/100 was divided into three equal parts thus defining two additional suprathreshold temperatures (SHP 1 and 2) which were delivered twice and in random order. The patients were asked to rate the perceived pain intensity immediately following each stimulus. The mean value of the two pain ratings for each temperature was calculated and used to plot the stimulus-response functions.

3.2.3 Compression-ischemia induced (differential) nerve block

To study which nerve fibre population that contributes to pain at 1 and 10 s prodding of the skin with von Frey filaments (Study II) and to DMA and DMD (Study III) a compression/ischemia-induced (differential) nerve block approach was used (Gasser and Erlanger, 1929; Ochoa and Yarnitsky, 1993). This was obtained through inflation of a sphygmomanometer cuff proximally placed around the symptomatic limb, inflating it to a level of 80 to 100 mm Hg above the systolic blood pressure (Ochoa and Yarnitsky, 1993). Shortly after inflating the cuff all patients experienced spontaneous non-painful paresthesias in the painful limb. This sensation vanished after a few minutes. The patients were carefully instructed not to move the limb during the course of the block since this may induce paresthesias or pain which may affect the outcome of the testing procedure.

During the nerve block single perception levels to cold (CL) and warmth (WL) were assessed every 1-3 minutes in the control area. This was done to monitor function in A-delta (cold) and C-fibres (warmth) during progression of the block. A significant elevation of a thermal perception level during the nerve block was defined as a sustained increase of at least 2 standard deviations (SD) compared to the individual pre-block mean. Also, the perception magnitude from brushing (normal, increased, decreased or none) the skin with a camel's hair brush (three times over the length of about 2 cm with a velocity of approximately 2 cm/s) in the control area compared to the contralateral pain free area (in patients with PNeP) or compared to an area just proximal to the cuff (in patients with CPSP) were assessed at the same intervals to monitor A-beta-fibre function (touch). The nerve block was terminated if the patient did not tolerate the pain caused by the cuff, if the spontaneous ongoing pain in the limb became unbearable, if a total loss of touch- and cold sensation indicating block of all A-fibres was obtained or at a maximum blocking time of 45 minutes. If the nerve block was terminated before significant elevation of CL and WL was obtained the time point of termination was assigned as the time point for elevation of CL and WL to allow for group level statistical analysis.

3.2.3.1 Study II

During the block single von Frey pain perception levels to 1 s (vF1) and 10 s (vF10) stimulation were repeatedly assessed every 1 – 3 minutes in the neuropathic area by single ascending stimuli. An increase in the pain perception level to von Frey filament stimulation of at least 2 steps (logarithmical increase) of the bending threshold during the block compared to pre-block values was regarded a significant increase.

3.2.3.2 Study III

3.2.3.2.1 Patients with PNeP

To assess the perceptual details of brush-induced DMA/DMD the patient was explicitly asked to describe the perception from brushing the skin with a camel's hair brush in the neuropathic area (three times over the length of about 2 cm with a velocity of approximately 2 cm/s) by choosing from the descriptors painful, unpleasant, normal touch or no sensation every 1 – 3 minutes during the block.

3.2.3.2.2 Patients with CPSP

In patients with CPSP an indirect method was employed to monitor progression of the nerve block compared to in patients with PNeP. Since all patients with CPSP had a lesion to the spino-thalamo-cortical pathway it was not possible to monitor progression of a nerve block in the painful limb in all patients due to sometimes marked impairment of temperature sensibility. Thus the nerve block was first performed in the non-painful contralateral limb to get a 'time table' of when the different types of nerve fibres were affected. The sensation to brushing in the contralateral control area during the block was compared to an area just proximal to the cuff. The time to loss of sensation to touch in the control area and to elevation of CL and WL were recorded. After conducting the nerve block on the non-painful side there was a recess of at least 30 minutes to allow for paresthesiae after the nerve block to disappear. In patients with pain located in the hand (n=6) the recess was necessary to allow for the non-painful hand to regain full motor function. The nerve block was then performed in the painful limb and the perception of brush-induced DMA/DMD was assessed in the painful area every 1 – 3 minutes (see above). The protocol was finally completed with data from the previous nerve block in the non-painful limb, i.e., time to loss of sensation to touch and time to elevation of CL and WL. The time line of the experimental procedure is presented in Fig.1**.** An illustrative case is depicted in Fig. 2.

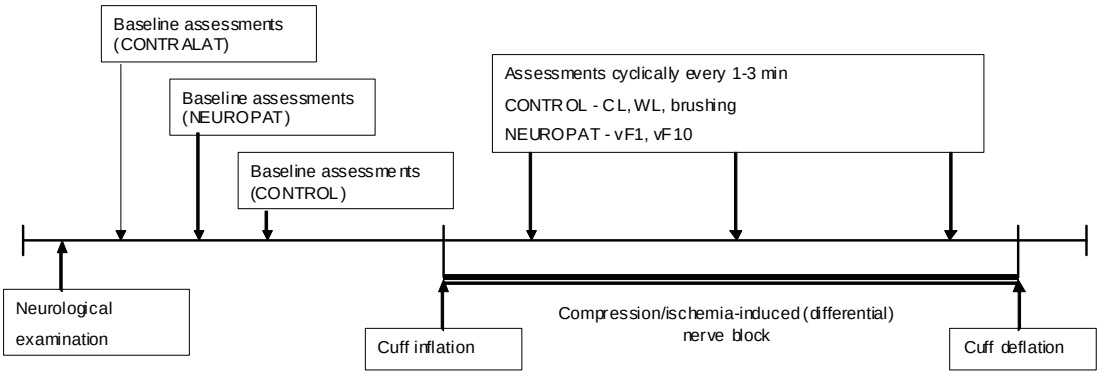

Fig. 1. Time course of the experiment. CONTRALAT, contralateral pain-free area; NEUROPAT, neuropathic area; CONTROL, control area neighbouring the painful site; CL, perception level to cold; WL, perception level to warmth; vF1, pain perception level to 1 s von Frey filament stimulation; vF10, pain perception level to 10 s von Frey filament stimulation.

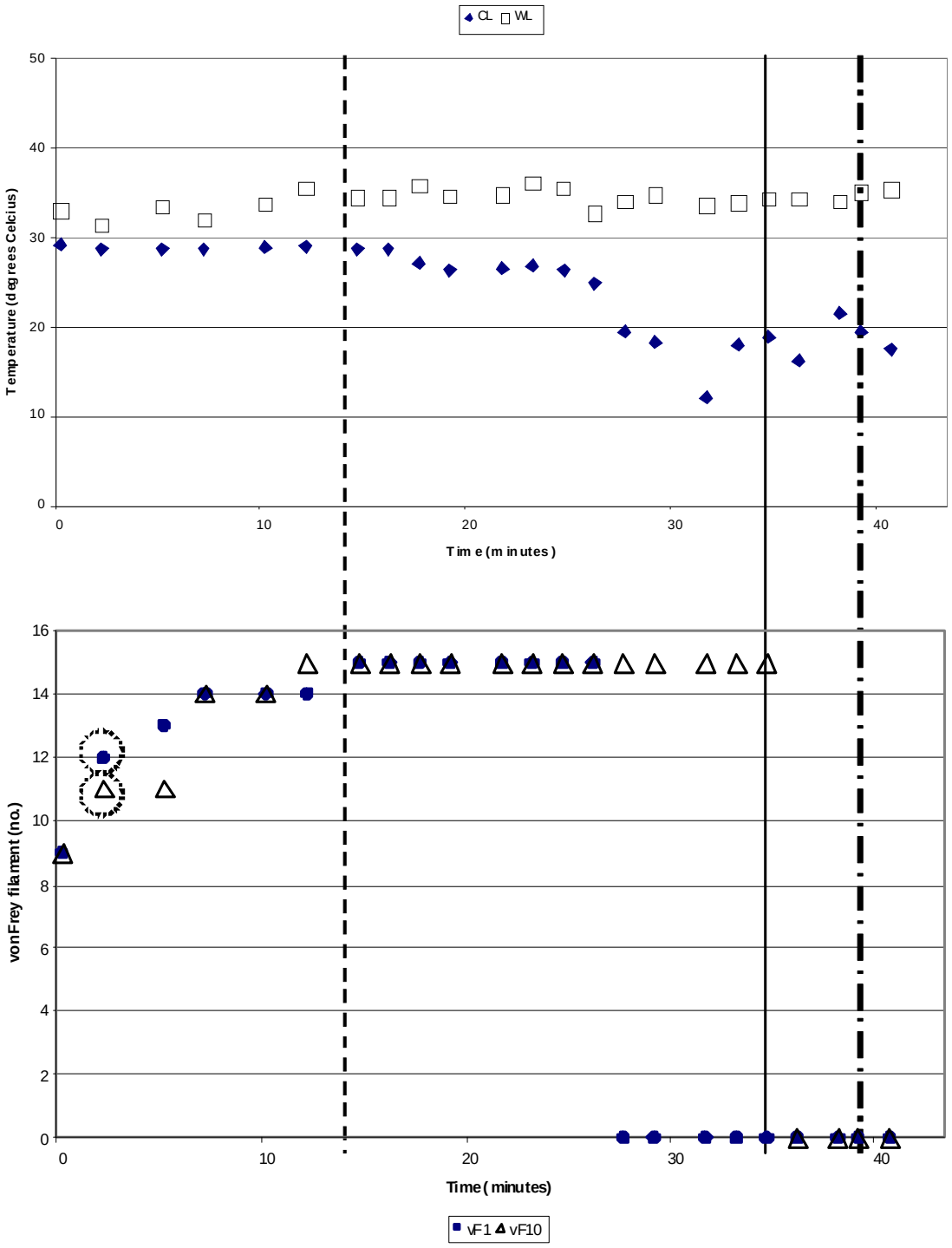

Fig. 2. Illustrative case of a patient during the compression/ischemia block.
CL, perception level to cold; WL, perception level to warmth; vF1, pain perception level to 1 s von Frey filament stimulation; vF10, pain perception level to 10 s von Frey filament stimulation.
(___), loss of sensation to touch in the control area
(----), elevation of CL in the control area
(-.-.-), elevation of WL in the control area
◌, elevation of vF1 or vF10

3.3 STATISTICS

Statistical significance was accepted at $p<0.05$. Data was analysed using Statistica 8.0, StatSoft®, Inc. Tulsa OK, USA.

3.3.1 Study I

Since most data variables had a non-symmetric distribution non-parametric statistics were applied. The test results from each side were compared within each patient group to probe intra-group side differences and then the calculated side-to-side difference was used to compare the two groups of patients. The difference between the two sides was calculated as the mean value obtained from the injured side – mean value from the contralateral side Analysis of the difference between sides in each group separately was performed by the Sign Test (Siegel and Castellan, 1988) (non-parametric data). The difference between sides for each parameter within each group was compared between the two patient groups using the Mann-Whitney *U* Test. The analysis of the stimulus-response functions for suprathreshold heat pain was made by calculating individual linear regression. The individual regression coefficients were then analysed within and between groups using Sign Test and the Mann-Whitney *U* Test. Data is presented as median and inter-quartile range.

3.3.2 Study II

Data was normally distributed. Results were analysed using a one-way ANOVA with repeated measures on factor "time" with 3 or 4 levels (time to elevation of vF1 and/or vF10, CL and WL). If the F-ratio for the main effect of "time" was significant, Fisher's LSD test or Tukey test was performed depending on the number of factor levels. When comparing patients with and without SMA data was analysed using a two-way ANOVA with repeated measures with the between-group factor 'SMA' with 2 levels (yes/no) and the within-group factor 'time' with 3 levels (time to elevation of vF1, CL and WL). If a significant interaction between 'SMA' and 'time' was found, simple main effects tests were performed, i.e., effects of one factor holding the level of the other factor fixed. If no significant interaction with factor 'time' was present and the F-ratio for the main effect of 'time' was significant, Fisher's LSD test was performed. The sphericity assumption was met in all the ANOVA models. T-test was used comparing time to elevation of vF10 in patients with SMA with time to elevation of vF1 in patients without SMA.

3.3.3 Study III

Data was normally distributed. Results were analysed using a one-way ANOVA with repeated measures on factor 'time' with 4 levels (time to transition/loss of DMA, time to elevation of CL and WL and time to loss of touch in the control area). If the F-ratio for the main effect of 'time' was significant Tukey test was performed. When comparing patients with PNeP and CPSP data was analysed using a two-way ANOVA with repeated measures with the between-group factor 'group' with 2 levels (PNeP/CPSP) and the within-group factor 'time' with 4 levels (time to transition/loss of DMA, time to elevation of CL and WL and time to loss of touch in the control area). In case of a significant interaction between 'group' and 'time', simple main effects tests

were performed, i.e., effects of one factor holding the other factor fixed. The sphericity assumption was met in all the ANOVA models.

3.3.4 Study IV

Results were analysed using a one-way ANOVA with repeated measures on factor 'side' with 3 levels (the injured side before and after SCS as well as the contralateral side before SCS). If the F-ratio for the main effect of 'side' was significant, post hoc analysis using Fisher's LSD test or t-test was performed. Due to skewed distribution for LTT and ΔCT these data have been log-transformed in order to meet the requirements for an adequate ANOVA. Due to a ceiling/floor effect of the variables PPT, SHP and CPT a non-parametric statistic approach with Mann-Whitney *U*-test and Friedman ANOVA was used. The correlation between the degree of change in perception threshold level on the injured side after SCS versus the degree of pain relief induced by SCS was calculated for each parameter using Spearman rank order correlation.

4 RESULTS

4.1 STUDY I

4.1.1 Side comparisons of sensibility in patients with pain

Four patients in the pain group (nos. 5, 8, 10, 15) reported dynamic mechanical allodynia to brushing in the area of neuropathy and all experienced pain instead of touch as the first sensation when testing with von Frey filaments. Hence LTT data is missing in these patients. When assessing the PPT on the painful side all 4 patients reported pain when placing the pressure algometer device against the skin and PPT could therefore not be assessed. Three patients were treated with a spinal cord stimulator and were requested to switch it off at least 12 hours before examination to eliminate the pain relieving effect.

Table 6 summarizes the outcome of QST on a group level. Patients with pain presented with an increased threshold to warmth (ΔWT) on the injured side but no difference could be demonstrated for the perception threshold to cold (ΔCT). There was an increased sensitivity to cold pain and pressure pain with decreased thresholds to CPT and PPT on the injured side but no difference could be demonstrated regarding HPT, SHP 60/100 or LTT. No difference between sides regarding stimulus-response function for suprathreshold heat pain could be demonstrated (p=0.75). Individual QST data is presented in Table 7.

Table 6.
Thermal and mechanical perception thresholds in the pain group (n=18) and in the pain free group (n=16).

Test parameter	Group	No. of tested patients	Contralateral area	Nerve injured area	*p* value
Thermal					
ΔWT/ºC	Pain	18	3.2 [2.1 ; 5.1]	4.1 [2.8 ; 10.7]	0.024*
	Pain free	16	1.8 [1.5 ; 2.4]	2.5 [2.0 ; 5.3]	0.001*
ΔCT/ºC	Pain	18	1.2 [1.0 ; 1.6]	1.7 [0.9 ; 3.0]	0.24
	Pain free	16	1.3 [1.1 ; 1.6]	1.7 [1.1 ; 2.2]	0.039*
CPT/ºC	Pain	18	10.0 [10.0 ; 18.7]	23.4 [14.4 ; 28.5]	0.003*
	Pain free	16	10.0 [10.0 ; 19.8]	17.5 [10.0 ; 23.2]	0.07
HPT/ºC	Pain	18	44.8 [43.3 ; 47.4]	42.6 [36.6 ; 48.1]	0.48
	Pain free	16	43.1 [40.4 ; 46.1]	40.4 [38.4 ; 46.4]	0.30
SHP 60/100 /ºC	Pain	12	47.8 [45.0 ; 48.7]	47.3 [39.5 ; 49.1]	0.75
	Pain free	10	48.7 [47.8 ; 49.7]	46.1 [43.3 ; 48.7]	0.29
Mechanical					
LTT/g	Pain	18/14	0.318 [0.095; 0.607]	0.318 [0.168; 0.567]	0.27
	Pain free	16	0.050 [0.030 ; 0.124]	0.250 [0.151; 0.794]	0.002*
PPT/kPa	Pain	18/14	228 [130 ; 269]	101 [44 ; 187]	0.016*
	Pain free	16	276 [200 ; 392]	265 [142 ; 366]	0.21

Threshold values are presented as medians with [25th and 75th percentiles].
ΔWT, perception threshold to warmth, difference from skin temperature;
ΔCT, perception threshold to cold, difference from skin temperature;
CPT, perception threshold to cold pain; HPT, perception threshold to heat pain;
SHP 60/100, suprathreshold heat pain rated 60/100 on NRS;
LTT, perception threshold to light touch; PPT, pressure pain threshold;
g, gram; ˚C, degree Celsius; kPa, kilo Pascal
* , significant difference with $p< 0.05$ (Sign test), injured side compared to uninjured side.

Table 7.
Individual thermal and mechanical perception thresholds in the pain group (n=18).

Pat	LTT g Con	LTT g Inj	PPT kPa Con	PPT kPa Inj	ΔCT ºC Con	ΔCT ºC Inj	ΔWT ºC Con	ΔWT ºC Inj	CPT ºC Con	CPT ºC Inj	HPT ºC Con	HPT ºC Inj
1	0.61	0.32	462	197	2.4	13.7	11.6	14.6	10.0	10.0	44.7	50.0
2	0.61	0.17	218	87	1.3	0.9	4.2	9.0	10.0	28.5	48.2	38.8
3	0.13	0.25	242	187	1.1	0.9	4.4	1.8	10.0	22.2	46.2	38.5
4	1.26	3.10	204	435	1.9	21.5	5.1	18.5	10.0	10.0	44.2	50.0
5	0.32	#	269	#	1.1	3.0	2.1	3.4	10.0	30.1	46.2	34.3
6	0.32	0.32	288	114	1.2	1.9	5.8	11.4	10.0	10.0	36.8	48.1
7	0.61	0.03	105	72	1.5	0.9	10.2	4.1	26.1	30.7	47.4	45.8
8	0.18	#	247	#	1.6	2.9	3.2	3.4	18.7	26.5	43.3	35.8
9	0.09	0.57	207	42	1.2	2.1	8.4	8.4	22.9	24.5	47.4	44.5
10	0.61	#	238	#	1.0	0.8	2.8	2.8	28.0	28.8	43.1	33.6
11	0.10	0.39	138	44	0.9	1.3	2.2	3.1	22.7	26.9	44.5	42.6
12	0.09	0.17	299	162	0.4	1.1	0.6	1.5	10.0	10.0	44.6	42.5
13	0.32	0.53	92	209	1.8	2.2	3.1	6.0	17.2	14.4	48.3	49.3
14	0.61	0.22	130	28	1.0	1.5	2.0	15.3	10.0	20.5	40.3	44.7
15	0.03	#	340	#	1.0	0.8	1.7	1.6	10.0	32.4	49.4	38.0
16	0.03	0.16	105	48	1.1	0.9	1.1	1.9	14.7	19.7	44.8	36.6
17	0.28	0.61	238	180	2.6	3.6	3.5	10.7	10.0	21.0	45.5	48.3
18	0.61	12.2	63	15	1.6	3.9	2.5	4.0	17.7	27.5	34.7	35.3

ΔWT, perception threshold to warmth, difference from skin temperature;
ΔCT, perception threshold to cold, difference from skin temperature;
CPT, perception threshold to cold pain; HPT, perception threshold to heat pain;
SHP 60/100, suprathreshold heat pain rated 60/100 on NRS;
LTT, perception threshold to light touch; PPT, pressure pain threshold;
g, gram; ˚C, degree Celsius; kPa, kilo Pascal; #, missing data; Con, contralateral side; Inj, injured side.

4.1.2 Side comparisons of sensibility in patients without pain

The outcome of QST on a group level is summarized in Table 6. Patients without pain presented with increased perception thresholds to light touch (LTT), cold (ΔCT) and warmth (ΔWT) on the injured side but no difference could be demonstrated regarding painful thermal or mechanical parameters (CPT, HPT, SHP 60/100 or PPT). No difference between sides regarding stimulus-response function for suprathreshold heat pain could be demonstrated (p=0.18). Individual QST data is presented in Table 8.

Table 8.
Individual thermal and mechanical perception thresholds in the pain-free group (n=16).

Pat	LTT g Con	LTT g Inj	PPT kPa Con	PPT kPa Inj	ΔCT ºC Con	ΔCT ºC Inj	ΔWT ºC Con	ΔWT ºC Inj	CPT ºC Con	CPT ºC Inj	HPT ºC Con	HPT ºC Inj
19	0.10	0.82	722	673	2.3	2.6	2.6	6.1	10.0	10.0	50.0	50.0
20	0.05	0.23	193	148	1.2	1.1	1.8	2.2	13.0	15.8	40.1	37.5
21	0.03	1.06	338	191	1.1	1.1	1.7	2.5	10.0	27.2	36.0	38.0
22	0.03	0.13	159	136	0.9	0.9	1.3	1.4	19.2	23.8	40.6	38.7
23	0.03	0.08	362	442	1.1	2.4	1.5	4.5	10.0	18.2	44.2	40.0
24	0.32	0.83	290	206	1.3	1.9	1.7	6.3	15.3	16.7	46.0	49.1
25	0.32	0.32	409	292	1.3	1.4	2.0	1.6	10.0	10.0	42.8	40.3
26	0.03	0.26	205	349	1.2	1.5	2.4	3.4	10.0	24.6	46.2	44.1
27	0.06	0.49	195	121	1.4	1.1	1.5	2.0	20.3	26.6	38.6	38.1
28	0.03	0.24	258	270	1.4	2.4	2.0	2.3	23.2	22.5	43.2	40.5
29	0.07	0.04	408	280	1.1	1.6	1.4	2.0	10.0	10.0	46.5	47.6
30	0.06	0.17	434	260	1.7	2.0	2.4	3.9	10.0	22.3	40.2	37.5
31	0.16	0.17	376	423	1.7	1.8	5.6	9.5	20.6	20.7	45.7	45.1
32	0.03	1.12	252	87	1.0	1.1	1.3	1.9	20.4	16.8	42.9	44.6
33	0.15	0.77	140	61	1.2	1.8	1.3	2.4	10.0	10.0	41.6	39.6
34	0.03	0.03	262	383	1.8	2.9	2.6	6.5	10.0	10.0	48.0	50.0

ΔWT, perception threshold to warmth, difference from skin temperature;
ΔCT, perception threshold to cold, difference from skin temperature;
CPT, perception threshold to cold pain; HPT, perception threshold to heat pain;
SHP 60/100, suprathreshold heat pain rated 60/100 on NRS;
LTT, perception threshold to light touch; PPT, pressure pain threshold;
g, gram; ˚C, degree Celsius; kPa, kilo Pascal; Con, contralateral side; Inj, injured side.

4.1.3 Side-to-side differences of sensibility between groups

No significant side-to-side difference of any of the mechanical or thermal perception thresholds or stimulus-response function for suprathreshold heat pain could be demonstrated between the two groups of patients with and without pain (Table 9).

Table 9.
Comparisons of side-to side differences of thermal and mechanical quantitative sensory testing in patients with (n=18) and without pain (n=16) after nerve injury.

Test parameter	Pain group	Pain free group	*p* value
Thermal			
Diff ΔWT/ºC	1.1 [0.0 ; 4.8]	0.9 [0.5 ; 3.3]	0.80
Diff ΔCT/ºC	0.6 [-0.2 ; 1.3]	0.3 [0.1 ; 0.6]	0.43
Diff CPT/ºC	5.2 [0.0 ; 11.8]	0.0 [-0.4 ; 6.9]	0.22
Diff HPT/ºC	-2.3 [-7.9 ; 4.5]	-0.6 [-2.8 ; 1.9]	0.47
Diff SHP 60/100/ºC	-0.7 [-8.5 ; 4.3]	-2.5 [-3.6 ; 0.3]	0.92
Mechanical			
Diff LTT/g	0.124 [-0.289; 0.330]	0.197 [0.031; 0.565]	0.42
Diff PPT/kPa	-76 [-137; -48]	-62 [-123; 30]	0.38

Diff, side-to-side differences presented as medians with [25th and 75th percentiles], obtained by subtracting the uninjured side from the injured side.
ΔWT, perception threshold to warmth, difference from skin temperature;
ΔCT, perception threshold to cold, difference from skin temperature;
CPT, perception threshold to cold pain; HPT, perception threshold to heat pain;
SHP 60/100, suprathreshold heat pain rated 60/100 on NRS;
LTT, perception threshold to light touch; PPT, pressure pain threshold;
g, gram; ˚C, degree Celsius; kPa, kilo Pascal

4.2 STUDY II

Nine patients reported concomitant SMA in the area of DMA. Six patients had ongoing pharmacological treatment of their neuropathic pain. Four patients were treated with a spinal cord stimulator.

4.2.1 Pain perception threshold to von Frey filament stimulation

In the neuropathic area all patients reported pain from the 1 s von Frey stimulation (Table 10). Only patients with clinically established SMA (n=9) reported sustained pain during the von Frey filament stimulation of 10 s (Table 10). Patients with only DMA reported pain during the initial 1 – 3 s of the total stimulus duration of 10 s and for a few s after the filament was removed.

Table 10.
Details of changes in mechanical pain perception levels during the nerve-block.

Pat	vF1 pre-block (g)	Time to elev. of vF1 (min)	vF1 elev. (g)	CL	WL	vF10 pre-block (g)	Time to elev. of vF10 (min)	vF10 elev. (g)	CL	WL
1	4.50	17.0	10.0	↑	→	-	-	-	-	-
2	4.50	3.5	10.0	→	→	-	-	-	-	-
3	0.225	4.5	0.82	↑	→	1.54	12.0	4.50	↑	→
4	0.225	3.0	30.0	→	→	-	-	-	-	-
5	0.45	5.0	1.54	→	→	-	-	-	-	-
6	2.65	2.0	10.0	→	→	2.65	2.0	7.0	→	→
7	4.50	3.5	22.0	↑	→	4.50	3.5	22.0	↑	→
8	0.03	6.0	0.45	→	→	0.03	6.0	0.45	→	→
9	0.82	6.5	2.65	→	→	0.45	14.0	1.54	→	→
10	2.65	3.0	10.0	→	→	-	-	-	-	-
11	0.45	2,0	22.0	→	→	0.45	2.0	22.0	→	→
12	4.50	8.0	10.0	→	→	4.50	8.0	10.0	→	→
13	15.0	16.0	30.0	→	→	15.0	10.0	30.0	→	→
14	0.125	3.0	4.50	→	→	0.125	3.0	7.0	→	→
15	7.0	8.0	15.0	→	→	-	-	-	-	-
16	0.07	3.0	0.45	→	→	-	-	-	-	-
17	0.45	4.0	2.65	→	→	-	-	-	-	-
18	0.225	7.0	1.54	→	→	-	-	-	-	-

(→), unaltered thermal perception level; (↑), elevation of thermal perception level;
(-), not applicable in patients without static mechanical allodynia;
vF1 elev., elevation of the 1 second von Frey pain perception level; vF10 elev., elevation of the 10 seconds von Frey pain perception level; CL, perception level to cold at elevation of F1/vF10; WL, perception level to warmth at elevation of vF1/vF10.

4.2.2 Time to threshold elevation of vF1

In the one-way ANOVA, there was a significant difference between time to elevation of vF1, CL and WL (n=18) during the differential nerve block ($F(2, 34) = 37.65$, $p<0.001$). In the post hoc analysis using Fisher's LSD test, elevation of vF1 occurred significantly prior to elevation of both CL ($p<0.001$) and WL ($p<0.001$) (Fig. 3). Comparing patients with (n=9) and without SMA (n=9) in the two-way ANOVA, no significant main effect of group could be demonstrated ($F(1, 16) = 0.02$, $p=0.89$). There was a significant difference between time to elevation of vF1, CL and WL during the differential nerve block within each group ($F(2, 32) = 36.02$, $p<0.001$). This analyses did not reveal a significant interaction ($F(2, 32) = 0.26$, $p=0.77$) between the factors 'time' (time to elevation of vF1, CL and WL) and 'group' (SMA yes/no) and thus no significant difference in time to elevation of vF1 between patients with and without SMA could be demonstrated ($p=0.77$) (Table 11). In the post hoc analysis

using Fisher's LSD test, time to elevation of vF1 occurred significantly prior to both time to elevation of CL (p<0.001) and WL (p<0.001) (Fig. 4). At the time of vF1 elevation 3/18 patients (nos. 1, 3, 7) presented with an elevated CL of ≥2 SD (an increase of 3.5 ˚C, 1.8 ˚C and 1.3˚C, respectively). None of the patients reported altered perception level to warmth at the time of elevation of vF1 (Table 10).

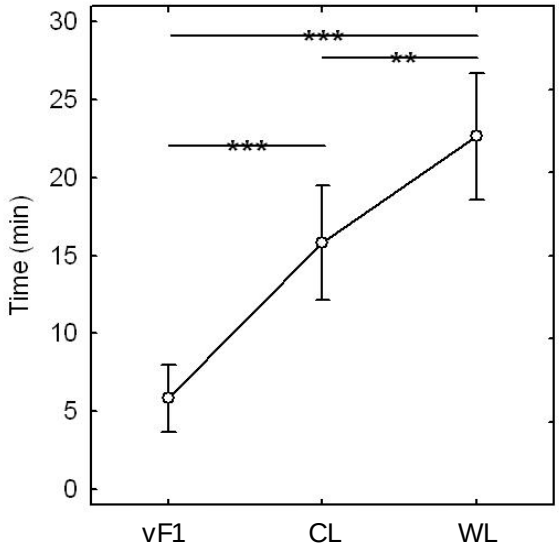

Fig. 3. The relationship between time to elevation of vF1, CL and WL (n=18). Mean time to elevation and 95 % confidence intervals are presented. In the one-way ANOVA significant differences are indicated by p-values in the figure (** p<0.01, *** p<0.001). vF1, pain perception level to 1 s von Frey filament stimulation; CL, perception level to cold; WL, perception level to warmth.

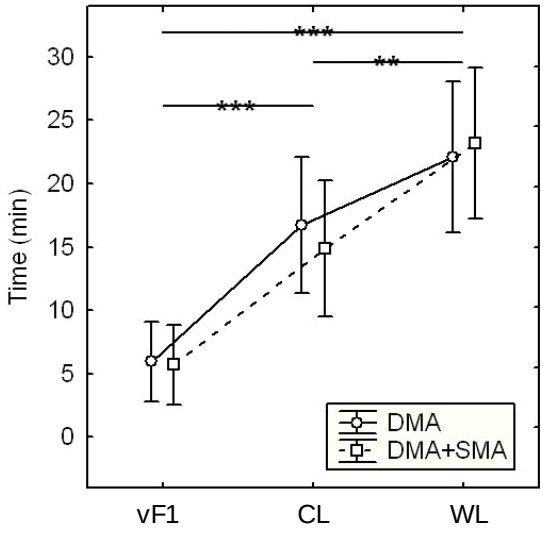

Fig. 4. The relationship between presence of SMA and time to elevation of vF1, CL and WL in patients with DMA only (n=9) and in patients with concomitant SMA (n=9) Mean time to elevation and 95 % confidence intervals are presented. In the two-way ANOVA significant differences are indicated by p-values in the figure (** p<0.01, *** p<0.001). vF1; pain perception level to 1 s von Frey filament stimulation; CL, perception level to cold; WL, perception level to warmth.

4.2.3 Time to threshold elevation of vF10

In the one-way ANOVA, there was a significant difference between time to elevation of vF1, vF10, CL and WL in patients with SMA (n=9) during the differential nerve block ($F(3, 24) = 17.32$, $p<0.001$). In the post hoc analysis using Tukey test elevation of vF10 occurred significantly prior to both elevation of CL ($p<0.05$) and WL ($p<0.001$) (Fig. 5). At the time of elevation of vF10, 2/9 patients (nos. 3, 7) presented with an elevated cold perception level of ≥ 2 SD (an increase of 3.4 ˚C and 1.3 ˚C, respectively). None of the patients reported altered perception level to warmth at the time of elevation of vF10 (Table 10).

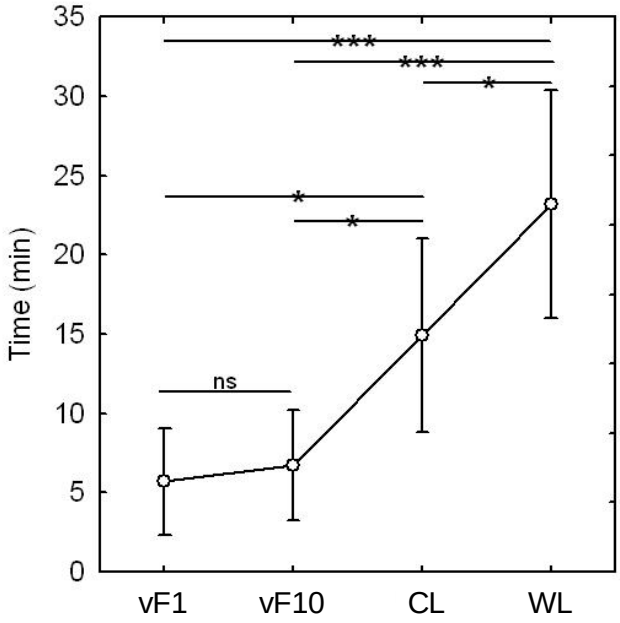

Fig. 5. The relationship between time to elevation of vF1, vF10, CL and WL in patients with DMA and concomitant SMA (n=9).
Mean time to elevation and 95 % confidence intervals are presented.
In the one-way ANOVA significant differences are indicated by *p*-values in the figure (* $p<0.05$, *** $p<0.001$).
ns, non-significant; vF1, pain perception level to 1 s von Frey filament stimulation; vF10, pain perception level to 10 s von Frey filament stimulation; CL, perception level to cold; WL, perception level to warmth.

4.2.4 The relationship between vF1 and vF10

There was no significant difference in time to elevation of vF10 in patients with SMA compared to time to elevation of vF1 in patients with SMA (p=0.98, one-way ANOVA, Tukey test) or compared to time to elevation of vF1 in patients without SMA (p=0.72, t-test) (Table 11).

Table 11.
Relationship between time to elevation of vF1 and vF10.

	vF1 DMA	**vF1 SMA**	**vF10 SMA**
vF1 DMA		NS, p=0.77[1]	NS, p=0.72[2]
vF1 SMA			NS, p=0.98[3]

vF1, 1 s von Frey pain perception level; vF10, 10 s von Frey pain perception level; DMA, patients with dynamic mechanical allodynia only; SMA, patients with dynamic and static mechanical allodynia; NS, non-significant; [1] = Result of two-way ANOVA, Fisher's LSD test; [2] = Result of t-test; [3] = Result of one-way ANOVA, Tukey test.

4.3 STUDY III

Six patients with PNeP and all of the patients with CPSP had ongoing pharmacological treatment of their neuropathic pain. Four patients with PNeP were treated with a spinal cord stimulator.

4.3.1 Patients with PNeP and DMA

During the compression/ischemia-induced differential nerve block all patients with PNeP experienced a transition of DMA to DMD before complete loss of sensation to brushing in the neuropathic area. In the one-way ANOVA, there was a significant difference between time to transition of DMA to DMD, time to elevation of CL and WL, respectively, and time to loss of brushing in the control area (equal to a complete A-beta fibre block) during the nerve block ($F(3, 51) = 23.49$, p<0.001). In the post hoc analysis using Tukey test, transition of DMA to DMD occurred significantly prior to elevation of both CL (p<0.05) and WL (p<0.001) as well as before loss of sensation to brushing in the control area (p<0.001) (Fig. 6).
At the time of transition of DMA to DMD 5/18 patients (nos. 1, 3, 7, 14, 17) presented with an elevated CL of ≥2 SD (an increase of 3.7 ˚C, 1.8 ˚C, 1.3˚C, 2.7 ˚C, 0.2 ˚C, respectively). One patient (no. 14) reported an elevation of the perception level to warmth of ≥2 SD (an increase of 5.7˚C) at the time of transition of DMA to DMD (Table 12).

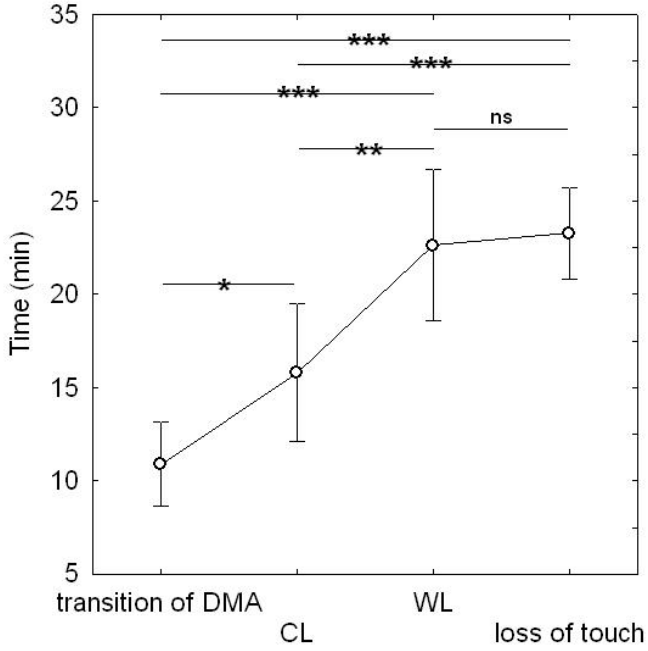

Fig 6. The relationship between time to transition of DMA to DMD, time to elevation of CL and WL and time to loss of brushing in the control area in patients with PNeP. Mean time to elevation and 95 % confidence intervals are presented.
In the one-way ANOVA significant differences are indicated by *p*-values in the figure (* $p<0.05$, ** $p<0.01$, *** $p<0.001$).
DMA, dynamic mechanical allodynia; DMD, dynamic mechanical dysesthesia; CL, perception level to cold; WL, perception level to warmth; PNeP, peripheral neuropathic pain.

Table 12.
Thermal sensory status at transition of DMA to DMD in patients with PNeP.

Patient	Time to transition (min)	CL at time of transition	WL at time of transition
1	19.0	↑	→
2	13.0	→	→
3	7.0	↑	→
4	10.5	→	→
5	11.0	→	→
6	7.0	→	→
7	3.5	↑	→
8	13.0	→	→
9	9.5	→	→
10	11.0	→	→
11	5.0	→	→
12	12.0	→	→
13	14.0	→	→
14	21.0	↑	↑
15	14.0	→	→
16	6.0	→	→
17	11.0	↑	→
18	9.0	→	→

DMA, dynamic mechanical allodynia; DMD, dynamic mechanical dysesthesia; PNeP, peripheral neuropathic pain; (→), unaltered thermal perception level; (↑), elevation of thermal perception level; CL, perception level to cold; WL, perception level to warmth.

4.3.2 Patients with CPSP and DMA

During the compression/ischemia-induced differential nerve block only 3/7 patients with CPSP (nos. 22, 24, 25) experienced a transition from DMA to DMD during the block. The rest of the patients lost DMA without transition to DMD before complete loss of sensation to brushing in the neuropathic area. In the one-way ANOVA, there was a significant difference between time to transition/loss of DMA in the affected area, time to elevation of CL and WL, respectively, and time to loss of brushing in the control area during the nerve block ($F(3, 18) = 37.81$, $p<0.001$). In the post hoc analysis using Tukey test, transition/loss of DMA occurred significantly prior to elevation of WL ($p<0.001$) and before loss of sensation to brushing in the control area ($p<0.001$). No significant difference could be demonstrated between time to transition/loss of DMA and time to elevation of CL ($p=0.99$) (Fig. 7). At the time of transition/loss of DMA 3/7 patients (nos. 19, 20, 25) presented with an elevated CL of ≥2 SD (an increase of 1.9 ˚C, 0.6 ˚C and 2.0 ˚C, respectively). None of the patients reported an elevation of the perception level to warmth at the time of transition/loss of DMA (Table 13).

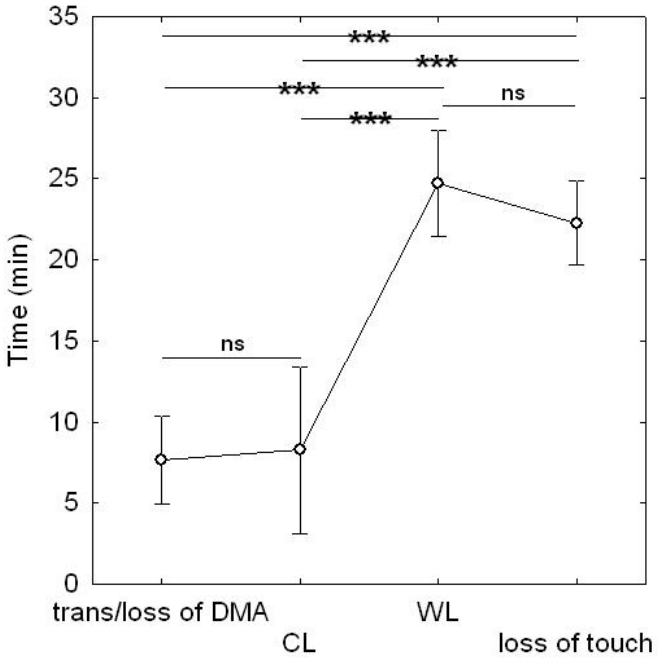

Fig. 7. The relationship between time to transition/loss of DMA in the affected area, time to elevation of CL and WL and time to loss of brushing in the control area in patients with CPSP. Mean time to elevation and 95 % confidence intervals are presented. In the one-way ANOVA significant differences are indicated by *p*-values in the figure (*** $p<0.001$).
DMA, dynamic mechanical allodynia; CL, perception level to cold; WL, perception level to warmth; CPSP, central post stroke pain; ns, non significant.

Table 13.
Thermal sensory status at transition of DMA to DMD or loss of DMA in patients with CPSP.

Patient	Transition from DMA to DMD	Loss of DMA without transition	Time to transition or loss of DMA (min)	CL at time of transition or loss of DMA	WL at time of transition
19		Yes	12.0	↑	→
20		Yes	6.0	↑	→
21		Yes	9.5	→	→
22	Yes		6.0	→	→
23		Yes	8.0	→	→
24	Yes		9.0	→	→
25	Yes		3.0	↑	→

CPSP, central post stroke pain; DMA, dynamic mechanical allodynia; DMD, dynamic mechanical dysesthesia; CL, perception level to cold; WL, perception level to warmth; (→), unaltered thermal perception level; (↑), thermal perception level elevation.

4.3.3 Comparison of patients with PNeP and CPSP

Comparing patients with PNeP (n=18) and CPSP (n=7) in the two-way ANOVA there was no significant difference between time to transition/loss of DMA (p=0.09), time to elevation of WL (p=0.53) or time to loss of sensation to brushing in the control area (p=0.62). However, a significant main effect of group could be demonstrated (F (3, 69) = 3.48, p=0.020) and time to elevation of CL during the differential nerve block in patients with CPSP occurred significantly prior to an elevation in patients with PNeP (p=0.024) (Fig. 8).

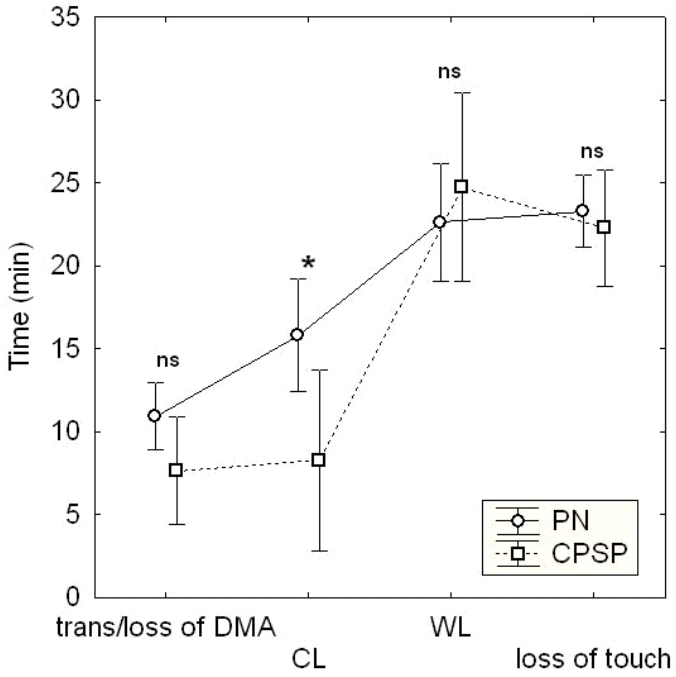

Fig. 8. The relationship between time to transition/loss of DMA, time to elevation of CL and WL and time to loss of brushing in the control area in patients with PNeP (n=18) and in patients with CPSP (n=7) (control area in the same limb in PNeP patients and in the contralateral limb in CPSP patients).
Mean time to elevation and 95 % confidence intervals are presented. In the two-way ANOVA significant differences are indicated by p-values in the figure (* p<0.05).
DMA, dynamic mechanical allodynia; CL, perception level to cold; WL, perception level to warmth; PNeP, peripheral neuropathic pain; CPSP, central post stroke pain; ns = non significant.

4.4 STUDY IV

4.4.1 Perception threshold to light touch

LTT was significantly increased on the injured side compared to the control side before stimulation ($p<0.001$) (Table 14). After SCS there was a significant decrease in LTT on the injured side compared to before SCS ($p<0.01$) (Table 15) but it was not completely normalized. After SCS the LTT was still significantly increased on the injured side compared to the uninjured side ($p<0.01$) (Table 16).

Table 14.
Sensory perception thresholds on the injured side compared to the uninjured contralateral side before SCS.

Test parameter	Contralateral side	Injured side	*p*-value
Thermal			
ΔWT/ºC	4.4 [2.2 ; 9.7]	9.8 [4.0 ; 17.6]	$p<0.01$ (2)
ΔCT/ºC	1.8 [1.3 ; 2.5]	3.2 [1.5 ; 6.8]	$p<0.001$ (2)
CPT/ºC	11.6 [10.0 ; 16.0]	10.0 [10.0 ; 23.2]	$p=0.98$ (4)
HPT/ºC	44.5 [42.5 ; 48.4]	45.1 [39.3 ; 50.0]	$p=0.95$ (3)
SHP 60/100 /ºC	48.3 [46.4 ; 50.0]	50.0 [42.9 ; 50.0]	$p=0.91$ (4)
Mechanical			
LTT/g	0.48 [0.15 ; 0.91]	2.96 [0.61 ; 8.79]	$p<0.001$ (1)
PPT/kPa	216 [136 ; 273]	184 [32 ; 226]	$p=0.18$ (4)

Threshold values are presented as medians with [25th and 75th percentiles].
ΔWT, perception threshold to warmth, difference from skin temperature;
ΔCT, perception threshold to cold, difference from skin temperature;
CPT, perception threshold to cold pain; HPT, perception threshold to heat pain;
SHP 60/100, suprathreshold heat pain rated 60/100 on NRS;
LTT, perception threshold to light touch; PPT, pressure pain threshold;
g, gram; ˚C, degree Celsius; kPa, kilo Pascal; SCS, spinal cord stimulation.
(1) = result of one-way ANOVA, t-test
(2) = result of one-way ANOVA, LSD-test
(3) = result of one-way ANOVA
(4) = result of Friedman ANOVA by ranks

4.4.2 Pressure pain threshold

Three patients (nos. 4, 5, 11) all reported intense pain when placing the pressure algometer against the skin on the injured side (i.e., mechanical allodynia) and PPT could therefore not be assessed. These patients were assigned a "worst-rank" value of 1 (kPa) to allow for group level statistical analysis. Before SCS there was no significant difference in the PPT on the injured side compared to the contralateral side (Table 14). After SCS the PPT significantly increased on the injured side compared to the same side before SCS ($p<0.01$) (Table 15) but still no significant difference could be

demonstrated compared to the contralateral side before SCS (p=0.06) (Table 16). After stimulation PPT could be assessed in patients nos. 4, 5, 11, all presenting with mechanical allodynia before SCS.

Table 15.
Sensory perception thresholds on the injured side before and after SCS.

Test parameter	Injured side before SCS	Injured side after SCS	*p*-value
Thermal			
ΔWT/ºC	9.8 [4.0 ; 17.6]	8.8 [3.5 ; 12.8]	p=0.28 (2)
ΔCT/ºC	3.2 [1.5 ; 6.8]	2.1 [1.7 ; 4.7]	p=0.09 (2)
CPT/ºC	10.0 [10.0 ; 23.2]	11.8 [10.0 ; 23.4]	p=0.98 (4)
HPT/ºC	45.1 [39.3 ; 50.0]	45.8 [39.3 ; 49.7]	p=0.95 (3)
SHP 60/100 /ºC	50.0 [42.9 ; 50.0]	49.4 [44.3 ; 50.0]	p=0.91 (4)
Mechanical			
LTT/g	2.96 [0.61 ; 8.79]	1.45 [0.32 ; 3.36]	p<0.01 (1)
PPT/kPa	184 [32 ; 226]	252 [168 ; 345]	p<0.01 (4)

Threshold values are presented as medians with [25th and 75th percentiles].
ΔWT, perception threshold to warmth, difference from skin temperature;
ΔCT, perception threshold to cold, difference from skin temperature;
CPT, perception threshold to cold pain; HPT, perception threshold to heat pain;
SHP 60/100, suprathreshold heat pain rated 60/100 on NRS;
LTT, perception threshold to light touch; PPT, pressure pain threshold;
g, gram; ˚C, degree Celsius; kPa, kilo Pascal; SCS, spinal cord stimulation.
(1) = result of one-way ANOVA, t-test
(2) = result of one-way ANOVA, LSD-test
(3) = result of one-way ANOVA
(4) = result of Friedman ANOVA by ranks

4.4.3 Non-noxious thermal perception thresholds

ΔCT was significantly increased on the injured side compared to the contralateral side before stimulation (p<0.001) (Table 14). Following SCS there was a non significant decrease in ΔCT on the injured side compared to before (Table 15). However, comparing the injured side after SCS with the uninjured side before SCS there was a normalisation of ΔCT and a significant difference compared to the contralateral side could no longer be detected (p=0.06) (Table 16). Two patients (nos. 1, 12) lacked perception of warmth on the injured side reporting pain as the first sensation during warm stimulation. This did not change after SCS and these patients were thus excluded from further statistical analysis. ΔWT was significantly increased on the injured side (n=14) compared to the contralateral side before stimulation (p<0.001) (Table 14). SCS did not induce any significant alteration of ΔWT on the injured side compared to the same side before stimulation (Table 15). Compared to the contralateral side ΔWT was still significantly increased (p<0.05) after SCS (Table 16).

Table 16.
Sensory perception thresholds on the injured side after SCS compared to the uninjured contralateral side before SCS.

Test parameter	Contralateral side before SCS	Injured area after SCS	*p*-value
Thermal			
ΔWT/ºC	4.4 [2.2 ; 9.7]	8.8 [3.5 ; 12.8]	$p<0.05$ (2)
ΔCT/ºC	1.8 [1.3 ; 2.5]	2.1 [1.7 ; 4.7]	$p=0.06$ (2)
CPT/ºC	11.6 [10.0 ; 16.0]	11.8 [10.0 ; 23.4]	$p=0.98$ (4)
HPT/ºC	44.5 [42.5 ; 48.4]	45.8 [39.3 ; 49.7]	$p=0.95$ (3)
SHP 60/100 /ºC	48.3 [46.4 ; 50.0]	49.4 [44.3 ; 50.0]	$p=0.91$ (4)
Mechanical			
LTT/g	0.48 [0.15 ; 0.91]	1.45 [0.32 ; 3.36]	$p<0.01$ (1)
PPT/kPa	216 [136 ; 273]	252 [168 ; 345]	$p=0.06$ (4)

Threshold values are presented as medians with [25th and 75th percentiles].
ΔWT, perception threshold to warmth, difference from skin temperature;
ΔCT, perception threshold to cold, difference from skin temperature;
CPT, perception threshold to cold pain; HPT, perception threshold to heat pain;
SHP 60/100, suprathreshold heat pain rated 60/100 on NRS;
LTT, perception threshold to light touch; PPT, pressure pain threshold;
g, gram; ˚C, degree Celsius; kPa, kilo Pascal; SCS, spinal cord stimulation.
(1) = result of one-way ANOVA, t-test
(2) = result of one-way ANOVA, LSD-test
(3) = result of one-way ANOVA
(4) = result of Friedman ANOVA by ranks

4.4.4 Noxious thermal stimulation

No significant difference in CPT, HPT or sensitivity to SHP could be demonstrated comparing the injured side before and after SCS as well as comparing the contralateral side before SCS with the injured side following SCS (Table 14-16).

4.4.5 The relationship between sensory thresholds and pain relief

There was no statistically significant correlation between the degree of threshold or suprathreshold alteration of any parameter in the neuropathic area following SCS compared to before stimulation versus the degree of pain relief induced by SCS.

5 DISCUSSION

5.1 SOMATOSENSORY FUNCTIONS AND SPONTANEOUS ONGOING PAIN (STUDY I)

Patients with painful unilateral partial peripheral traumatic nerve injury demonstrated a significant decrease in the perception threshold to cold pain and pressure pain (i.e., allodynia) on the painful side in conjunction with an increase in the perception threshold to non-painful warmth. In patients without pain there was a significant perception threshold increase to light touch, cold and warmth, on the injured side but no difference could be demonstrated regarding painful sensory modalities. However, when comparing side-to-side differences of sensory functions between groups of patients with and without pain no significant differences were found. This calls for cautious interpretation of our data.

Previous studies on peripheral traumatic nerve injury (Gottrup et al., 2000; Aasvang et al., 2008) included patients with possible confounding factors regarding the aetiology of pain. In this, we believe, comparatively homogenous patient group there were no obvious signs of neurogenic inflammation (Torebjork et al., 1992; Koltzenburg et al., 1994; Rowbotham and Fields, 1996) as an indication of possible peripheral sensitization. We can not rule out, however, the presence of facilitated transducer mechanisms to certain stimulus energies in groups of nociceptors.

Some sensory deficit is an anticipated sequel from loss of fibres as a result of nerve injury and a common feature of neuropathic pain conditions. In experimental models of traumatic peripheral nerve injuries axotomy not only causes deafferentation peripheral to the site of injury but also induces substantial retrograde transganglionic degeneration into the spinal cord of cutaneous sensory dorsal root ganglion (DRG) neurons (Ygge and Aldskogius, 1984; Hu and McLachlan, 2003), and hence loss of spinal cord input. Immediate nerve repair has been found not to completely prevent this neuronal degeneration but can to some extent increase the survival of sensory neurones (Welin et al., 2008). However, motor neurone loss can be almost totally eliminated and function restored by an early nerve repair (Ma et al., 2003). In the pain-free group in this study all patients had undergone nerve suturing and demonstrated sensory loss in more non-painful domains than patients with pain. This could indicate that nerve suturing may prevent increased excitability and the development of pain.

Results regarding sensory deficits from studies in patients with painful polyneuropathy are at variance with our current findings. In patients with HIV neuropathy Martin and co-workers reported a more pronounced impairment of C-fibre mediated innocuous warm perception thresholds in patients with pain than in pain-free patients (Martin et al., 2003). Patients with painful diabetic neuropathy demonstrated a significantly more pronounced impairment of non nociceptive thermal and vibration detection thresholds compared to patients without pain (Ziegler et al., 1988) suggesting a more pronounced loss of both small and large diameter fibres in patients with pain. However, Bouhassira

and co-workers found no significant difference between any of the thermal parameters in patients with and without pain after HIV neuropathy (Bouhassira et al., 1999).

Although not amounting to significant side-to-side differences between groups the disparate result on somatosensory functions in patients with and without pain could be signs of different inherent pain protective mechanisms in the two groups. The increase in excitability in nociceptive channels reflected by heat- and pressure allodynia in patients with pain may indicate a loss of pain regulatory mechanisms, although the level of such pathophysiology along the neuraxis is unknown and may have a bearing on not only stimulus-evoked pain but also the presence of spontaneous pain. Augmented stimulus-evoked pain has been reported by others from studies on painful neuropathy. In patients with post-mastectomy pain (Gottrup et al., 2000) and pain after unilateral inguinal herniotomy (Aasvang et al., 2008), allodynia to pressure and abnormal temporal summation to pinprick pain on the injured side compared to the normal side have been demonstrated, suggesting peripheral and/or central hyperexcitability contributing to, at least, stimulus-evoked pain. Decreased mechanical pain thresholds and increased responses to suprathreshold nociceptive mechanical stimulation were demonstrated also in patients with painful polyneuropathy due to HIV infection (Bouhassira et al., 1999).

Besides increased peripheral activity due to, e.g., ectopic impulse discharge and ephaptic transmission increase in spinal cord excitability has been suggested to be a built in compensation for some of the deficits in the afferent nociceptive drive after nerve injury (Chapman et al., 1998; Suzuki and Dickenson, 2000; Suzuki et al., 2000). Also, disinhibition of spinal neurons due to loss of peripheral input may come into play (Castro-Lopes et al., 1993; Moore et al., 2002). Increased spinal excitability induced by a peripheral nerve injury may thus compensate (or over-compensate) for or restore spinal responses to peripheral stimuli in spite of decreased afferent input. Variation in the degree of peripheral spontaneous activity, compensation or disinhibition anywhere along the neuraxis could provide an explanation to the development of spontaneous- and stimulus-evoked pain and also to the diverse somatosensory findings seen in patients with and without pain after peripheral nerve injury (Lindblom and Tegner, 1985; Hansson and Kinnman, 1996; Pertovaara, 1998). In this study, patients without pain presented with increased perception thresholds only to non-painful stimuli (i.e., hypoesthesia to light touch, warmth and cold) on the injured side compared to the non-injured side and would thus be devoid of protruding over-compensation mechanisms as part of a normal protective system against pain development after traumatic peripheral nerve injury.

Suprathreshold magnitude estimation of heat pain stimuli were included (Hansson and Lindblom, 1992; Vestergaard et al., 1995; Attal et al., 1999; Bouhassira et al., 1999) in order to challenge a perhaps more relevant part of the stimulus-response function of this pain channel. In the present study suprathreshold heat pain stimuli elicited similar responses in both patients with and without pain and no significant side-to-side difference was found. The lack of a detectable difference in heat pain threshold and magnitude estimation of suprathreshold heat pain may be explained by the relatively lesser need for spatial summation in the periphery with regard to this modality (Yarnitsky and Ochoa, 1991). This relatively independence on spatial summation may

be of phylogenetic importance since the sensation of heat pain is an important part of body protection to external energies. A detectable difference in function of this C-nociceptor channel due to loss of fibres subserving this sense therefore has to be substantial in order to be detectable.

5.2 SOMATOSENSORY FUNCTIONS AND MECHANICAL ALLODYNIA (STUDY II, III)

It is widely accepted that during a compression/ischemia induced nerve block conduction in myelinated fibres is blocked successively depending on thickness and starting at an early phase prior to unmyelinated fibres (Gasser and Erlanger, 1929; Sinclair and Hinshaw, 1950; Torebjork and Hallin, 1973). It has been claimed, although not observed in thesis work, that the sequence of blocking within the myelinated fibre group is insufficiently differentiated by such an approach as shown by the nearly simultaneous disappearance of the sensation of light touch (A-beta) and cold (A-delta) (Yarnitsky and Ochoa, 1989; Yarnitsky and Ochoa, 1991).

5.2.1 Dynamic and static mechanical allodynia (Study II)

Elevation of both vF1 in patients with DMA and vF10 in patients with SMA occurred concurrently in time and significantly prior to an increase in the perception level to cold during the continuous nerve block, pointing to the involvement of A-beta fibres as the peripheral substrate. Single patients demonstrated a slight decrease in cold perception levels at the time of elevation of vF1 or vF10 and a possible contribution to mechanical allodynia from A-delta-fibres can therefore not completely be ruled out although the recorded alterations were minor. None of the patients reported an elevation of the perception level to warmth at the time of elevation of vF1 or vF10 excluding contribution from C-fibres.

Further, only patients with clinically established SMA (n=9) reported continuous pain to a sustained 10 s von Frey filament stimulation (vF10). Patients with only DMA (n=9) reported pain merely for the initial 1 – 3 s of the total stimulus duration of 10 s and for a few seconds after the filament was lifted from the skin. In the study by Ochoa and Yarnitsky SMA in patients with neuropathic pain persisted in a majority (15/18) of patients during a compression/ischemia nerve block although diminished in intensity (in 10/15 patients) when loss of cold and touch sensation was established and warm sensation remained unaltered (Ochoa and Yarnitsky, 1993). The result was interpreted by the authors as an indication that SMA predominantly was mediated by C-fibres (Ochoa and Yarnitsky, 1993).

Regarding the possible involvement of A-delta fibres in mediating SMA in the present study we monitored cold-activated A-delta fibres during the block but did not explicitly test the function of A-fibre nociceptors, i.e., first pain to heat, mechanical or electrical stimuli. Since A-fibre nociceptors have been shown to be more resistant to a compression/ischemia nerve block than all other A-fibres some of their axons may still conduct after all tested A-fibre related functions (i.e., touch and cold) are blocked (Ziegler et al., 1999). However, A-fibre nociceptors seem not to be the main candidate as the peripheral substrate of SMA because the elevation of vF1 and vF10 occurred

early during the block when A-fibre nociceptors would be fairly resistant to compression/ischemia. This supports the role of A-beta fibres as peripheral mediators to both vF1 and vF10 in the von Frey stimulus range used in this study although different receptor organs may be involved, i.e., rapidly (RA) and slowly (SA-I) adapting mechanoreceptors, respectively.

The finding of A-beta fibre involvement in DMA lends support from previous experiments on patients with peripheral neuropathic pain indicating a crucial role for low threshold A-beta fibres in the generation of pain during light mechanical stimuli (Lindblom and Verrillo, 1979; Campbell et al., 1988; Price et al., 1989; Nurmikko et al., 1991; Ochoa and Yarnitsky, 1993). Other myelinated afferents than low threshold A-beta mechanoreceptive fibres may, however be implicated in DMA in patients with PNeP such as nociceptive A-beta fibres (Cain et al., 2001; Djouhri and Lawson, 2004) and A-delta low-threshold mechanoreceptors (Adriaensen et al., 1983). The involvement of C-fibre nociceptors with low mechanical threshold (Slugg et al., 2000) and low-threshold mechanoreceptive C-fibres (Vallbo et al., 1993; McGlone et al., 2007) seem less conceivable since C-fibres were unaffected during the continuous nerve block as judged by the preservation of warm perception at detection level.

Techniques to assess different allodynias at perception threshold level are in demand as adjuncts to suprathreshold stimuli in intervention studies aimed at modifying these stimulus-evoked phenomena (Samuelsson et al., 2005). Pain induced by usually non-painful von Frey filament prodding of the skin has been reported on in patients with neuropathic pain and may be a useful approach if the type of stimulation could be linked to activation of specific peripheral nerve fibres (Lindblom and Hansson, 1991).

5.2.2 Dynamic mechanical allodynia and dysesthesia (Study III)

There was a transition of DMA to DMD during the compression/ischemia-induced nerve block in all patients with PNeP (n=18) and in 3/7 patients with CPSP. The remaining patients with CPSP lost DMA without transition to DMD. The transition of DMA to DMD or loss of DMA (in patients without transition) occurred early and concurrently in time during the nerve block and was paralleled by a continuous impairment of mainly A-beta fibre function suggesting DMA to be mediated via that group of fibres in both groups of patients. Single patients in both groups demonstrated a slight decrease in cold perception levels at the time of transition/loss of DMA and a possible contribution to DMA from A-delta fibres can therefore not completely be ruled out, although the recorded alterations were minor. Only one patient with PNeP and none of the patients with CPSP reported an elevation of the perception level to warmth at the time of transition/loss of DMA excluding a major contribution from C-fibres.

In patients with PNeP the transition from DMA to DMD occurred significantly prior to an increase in the perception level to cold but no significant difference between time to transition/loss of DMA and time to increase in the perception level of cold could be demonstrated in patients with CPSP. In patients with CPSP the control area used to monitor progression of the nerve block was located in the contralateral non-painful limb and it is conceivable that disturbances in attention induced by sensations

(paresthesias, pain) from the effect of the sphygmomanometer cuff could explain the early but small increase in the perception level to cold seen in 3/7 patients with CPSP already during the initial phase of the nerve block. This initial increase was not seen in patients with PNeP indicating that possible distraction from cuff related effects were minor in this group of patients where the nerve block was performed in an already painful limb. In patients with CPSP the lack of statistical significance on a group level between time to transition/loss of DMA and time to increase in the perception level of cold could also indicate a type II error since the study was performed in a comparatively small group of CPSP patients due to difficulties in recruiting patients fulfilling preset inclusion criteria.

The fact that the transition of DMA to DMD during the nerve block occurred when mainly only A-beta fibre function was affected indicates that also DMD has a peripheral substrate within the A-beta group. We therefore suggest DMA to be the hyperbole of DMD, the difference being the number of mechanoreceptive fibres having access to the nociceptive system in the periphery via ephaptic transmission or in the central nervous system.

5.3 SOMATOSENSORY FUNCTIONS AND PAIN RELIEF (STUDY IV)

Following SCS there was a significant decrease in the perception threshold to light touch and a significant increase in the pressure pain threshold in the neuropathic area compared to before SCS. Compared to the contralateral side these perception thresholds changed towards normalisation also including a significant normalisation of the perception threshold to non painful cold. SCS did not induce any significant alterations in sensitivity to noxious temperature stimulation. In addition, there was no significant correlation between the degree of threshold alterations of any mechanical- or thermal parameter versus the degree of pain relief induced by SCS.

Besides increased peripheral activity due to, e.g., ectopic impulse discharge and ephaptic transmission experimental animal models suggest a possible increase in spinal excitability following a peripheral nerve injury that might partially compensate (or over-compensate) for or even restore spinal responses to peripheral stimuli in spite of decreased afferent input (Chapman et al., 1998; Suzuki and Dickenson, 2000; Suzuki et al., 2000). This may provide an explanation as to the development of spontaneous- and stimulus-evoked pain and also to the diverse somatosensory findings seen in patients (Hansson and Kinnman, 1996). In the present study sensitivity to innocuous mechanical and thermal stimuli was significantly decreased on the injured side compared to the uninjured side before SCS but no difference could be demonstrated regarding painful mechanical or thermal stimulation. This is at variance with previous reports of increased mechanical and/or thermal pain sensitivity on the injured side in patients with post-mastectomy pain (Gottrup et al., 2000), pain after unilateral inguinal herniotomy (Aasvang et al., 2008) and pain after a variety of peripheral nerve injuries (Landerholm et al., 2010) (Study I). The contrasting results could in fact indicate that long term use of SCS may induce reversible or permanent changes in spinal excitability. This notion is supported by a study on patients with post amputation pain reporting decreased sensitivity to noxious and innocuous electrical stimulation after long-term use of SCS not seen in short-term stimulation during a test period (Doerr et al., 1978).

We found increased sensitivity to light touch and non-painful cold in conjunction with decreased sensitivity to pressure pain on the injured side following SCS induced pain relief. This is in accordance with previous reports of improved sensitivity of somatosensory function as a result of pain relief indicating a possible link to the release of a proposed functional block by a given pain relieving measure on somatosensory function induced by activity in the nociceptive system (Lindblom and Verrillo, 1979; Marchettini et al., 1992). The underlying mechanisms of such a functional block are not known. In addition, in the present study SCS did not induce any significant alterations of sensitivity to noxious thermal stimulation in the painful area which is consistent with findings from Eisenberg and co-workers (Eisenberg et al., 2006).

The lack of a significant correlation between the degree of sensory threshold changes and the degree of pain relief induced by SCS indicates that the observed sensory changes following SCS are mechanistically unrelated to pain relief. The previously reported positive correlation between decreased sensitivity to noxious thermal stimulation and pain relief following SCS demonstrated in patients with post surgical pain should be cautiously interpreted since the sensory testing was made in an area influenced by SCS-induced paresthesia but was located outside the painful area (Marchand et al., 1991). Hence, the outcome of that study cannot be compared to our results where sensory assessments were made within the painful area.

5.4 METHODOLOGICAL SHORTCOMINGS

5.4.1 Study I

There are obviously several possible explanations as to why no significant difference in any single parameter comparing side-to-side differences between groups was found in this study and certain shortcomings need consideration. The two groups differ with regard to the cause of nerve injury where all patients without pain had a clear partial cut injury as judged by visual inspection during surgery, and were sutured, while patients in the pain group are more heterogeneous. We can not rule out that this diversity could contribute to the non-significant differences in single parameters between groups. The optimal situation would be to compare patients with partial injuries with and without pain where all nerves were sutured or non-sutured. It deserves to be mentioned that there is no available information from human clinical studies about which nerves, pure sensory or mixed, that are more prone after injury to be the source of neuropathic pain. The fact that approximately half of the patients in the pain group had injuries to pure sensory nerves and the other half injuries to mixed nerves may indicate that development of neuropathic pain is not depending on the proportion of sensory nerve fibres in the injured nerve. None of the patients participating in this study had undergone neurophysiological examination as part of clinical routine work-up. Therefore, in the pain group the diagnosis of neuropathic pain can not be assessed with the highest degree of certainty, i.e., ‘definite’ neuropathic pain, according to the recently proposed grading system (Treede et al., 2008). Hence, we only claim that ‘probable’ neuropathic pain was at hand. A type II error must be considered since the study was performed in a comparatively small group of patients. A post-hoc power analysis revealed the need for increasing the sample size to several hundred patients to be able to demonstrate a possible difference in any parameter between groups. In support of this, the relatively large variability in pain thresholds found in healthy

subjects (Rolke et al., 2006) is a relevant observation. Such a study would be extremely time consuming and calls for the need of a multicenter design. We lack appropriate reference values for the employed QST tests in the multiple body regions that were examined. This would require a huge reference value data base from healthy subjects and is not available in our laboratory or in the literature. Moreover, comparing sensory function in the injured area with the contralateral homologous site in the individual patient is not possible since the minimum difference to be regarded as pathological is unknown. Based on this, no conclusions can be drawn on an individual level. Also, the distribution of sensory abnormalities within the innervation territory of the injured nerve might not be homogeneous and the assessments made in a restricted part of the neuropathic area may randomly pick up function not representative of the larger part of that area (Leffler and Hansson, 2008). In addition, if the pain generator is located in a neuroma proximal to the examination area the spontaneous activity is not likely to be reflected by the somatosensory profile within that area. Finally, and perhaps most importantly, altered sensory perception thresholds, especially non-nociceptive modalities, may not at all be related to pathophysiological mechanisms involved in spontaneous ongoing neuropathic pain after peripheral nerve injury.

5.4.2 Study II

Some methodological considerations deserve attention. The von Frey filaments used in this study did not evoke pain in the contralateral pain-free area or in the control area in any patient. However, activation of nociceptive somatosensory channels cannot be ruled out because numerous human and animal studies have shown that the used range of von Frey filaments is sufficient to activate both unmyelinated and myelinated nociceptors, however not necessarily giving rise to pain (Adriaensen et al., 1983; Schmidt et al., 1995; Andrew and Greenspan, 1999; Slugg et al., 2000). In addition, the used range of von Frey filaments increased logarithmically thereby providing a less detailed resolution of measurements in the higher stimulus range, i.e., up to 30 g. Also, the examination of different sensory modalities was made cyclically and approximately every 1 – 3 min during the nerve block. This range was allowed to secure cessation of stimulus-induced aftersensations in some patients and thus there is a possibility of perception level elevations occurring between examination intervals hence resulting in a recorded value of the time to perception level elevation higher than the true value. Furthermore, an increased perception level early on during the nerve block could be related to disturbances in attention induced by sensations (paresthesias, pain) from the effect of the sphygmomanometer cuff. However, in this study there was no initial increase of temperature perception levels during the first 5 minutes of the block indicating that distraction from cuff related effects were minor at least in the non-neuropathic skin area.

5.4.3 Study III

Some methodological issues should be considered. In patients with CPSP there is a possibility of biased patient selection criteria since all patients with pain in the upper limb (n=6) had to have spared motor function in the painful hand to be able to participate in the study. Hence all patients with CPSP had primarily sensory symptoms as a sequel of their lesion. Also, the examination of different sensory modalities was made cyclically and approximately every 1 – 3 min during the nerve block. This range

was allowed to secure cessation of stimulus-induced aftersensations in some patients and thus there is a possibility of perception level elevations occurring between examination intervals. Hence in patients with CPSP a possible transition from DMA to DMD and subsequent loss of DMD could have been missed in 4/7 patients. In patients with PNeP the recorded time to increase of CL could be higher than the true value resulting in a false significant difference between the time to transition of DMA to DMD and time to increase of CL. This possibility, however, seems less conceivable since only a few patients reported an increase of CL occurring close in time to transition of DMA to DMD.

5.4.4 Study IV

Some methodological considerations deserve attention. Since the primary aim of this study was to investigate the modulatory effect of SCS on somatosensory functions within the area of neuropathy and its correlation to relief of spontaneous pain examination of a control group consisting of patients reporting no pain relief by SCS was excluded. This strategy was also supported by the fact that long-term SCS may induce changes in spinal excitability possibly affecting the outcome of sensory testing (Doerr et al., 1978) not seen in short-term stimulation during a test period prior to permanent implantation of the SCS device. Also, in our setting patients with painful neuropathy and radiculopathy that fail to respond to SCS during the test period will not be eligible for permanent implant. Hence, a comparable control group is not available. In addition, the distribution of sensory abnormalities within the innervation territory of the injured nervous structure might not be homogeneous and assessments made in a restricted part of the neuropathic area may just randomly pick up function not representative of the larger part of that area (Leffler and Hansson, 2008). Further, to allow for group level statistical analysis several patients were assigned cut-off values or ‘worst-rank values’ for the tested parameters if they failed to respond during the examination. This could explain why no significant difference in any of the noxious thermal parameters was found comparing the injured side before and after SCS as well as the contralateral side before SCS and the injured side after stimulation since assignment of ‘worst-rank values’ were especially frequent when testing these parameters.

6 THESIS SUMMARY

6.1 STUDY I

In conclusion, increased pain sensitivity to cold and pressure was found on the injured side in pain patients, pointing to hyperexcitability in the pain system, a finding not verified by a more challenging analysis of side-to-side differences between patients with and without pain. To what extent the indications of hyperexcitability in the pain system contribute to spontaneous pain as a result of increased peripheral activity, disinhibition or facilitatory (over) compensation mechanisms, or combinations thereof, cannot be determined. Pain-free patients may possess an inherent resistance to respond with such alterations to loss of peripheral input thereby protecting them from pain development after nerve injury. Since only a fraction of patients with peripheral nerve injuries suffer from pain inborn pain protective mechanisms seems to be the normal condition in most individuals and the malfunction in these systems resulting in neuropathic pain after injury to be an exception.

6.2 STUDY II

In conclusion, it is proposed that pain induced by 1 s and 10 s von Frey filament stimulation at perception threshold in patients with neuropathy and DMA/SMA is predominantly mediated by activity in peripheral non-nociceptive A-beta mechano-receptors although different receptor organs may be involved, i.e., rapidly (RA) and slowly (SA-I) adapting mechanoreceptors, in DMA and SMA, respectively. The methods used to assess the perception thresholds of mechanical allodynias deserve further attention as possible adjuncts to suprathreshold stimuli in intervention studies aimed at modifying these stimulus-evoked phenomena.

6.3 STUDY III

In conclusion, DMA in patients with neuropathic pain is transferred to a dysesthetic sensation or lost without transition paralleled by a continuous impairment of mainly A-beta-fibre function during a compression/ischemia-induced differential nerve block. These findings point to DMA and DMD both being mediated via that group of fibres both in patients with PNeP and CPSP. Hence, dynamic mechanical allodynia is the hyperbole of dynamic mechanical dysesthesia despite the location of the lesion level along the neuroaxis the difference being the number of mechanoreceptive fibres having access to the nociceptive system.

6.4 STUDY IV

In conclusion, decreased perception threshold to light touch and increased perception threshold to pressure pain were found in the neuropathic area following SCS compared to before stimulation. Compared to the contralateral side these perception thresholds changed towards normalisation also including a significant normalisation of the perception threshold to non painful cold. These alterations indicate a possible link to the release of a functional block on somatosensory function induced by activity in the nociceptive system. The degree of the observed sensory changes following SCS was however unrelated to the degree of pain relief in the studied patient group.

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8 SAMMANFATTNING PÅ SVENSKA

Bakgrund och syfte: Patienter med neuropatisk smärta lider av spontansmärta och ibland även av stimulusutlöst smärta. Allodyni definieras som smärta utlöst av en normalt icke smärtsam stimulering. Dynamisk mekanisk allodyni är smärta utlöst av en lätt strykning över hudytan och statisk mekanisk allodyni framkallas av ett ihållande lätt tryck mot huden. Undergrupper av patienter rapporterar att dynamisk mekanisk allodyni kan variera i intensitet över tid och ibland endast upplevas som obehag (dysestesi). Avhandlingsarbetets syfte var att söka efter gemensamma nämnare för somatosensorisk funktion i det nervskadade området som kan kopplas till bakomliggande mekanismer för utveckling av eller skydd mot smärta efter traumatisk perifer nervskada (Studie I). Därutöver var avsikten att undersöka om kort eller längre icke smärtsamt tryck med von Frey filament mot huden i det nervskadade området kan användas för att mäta perceptionströsklar för dynamisk mekanisk och statisk mekanisk allodyni (Studie II). Dessutom var syftet att undersöka om dynamisk mekanisk allodyni är en förstärkning av dynamisk mekanisk dysestesi båda medierade av nervfibrer som i periferin förmedlar beröring (Studie III). Slutligen ville vi även studera den modulerande effekten av ryggmärsstimulering på somatosensorisk funktion inom det smärtande området hos patienter med perifer neuropatisk smärta (Studie IV).

Metod: Med metoder för kvantitativ känseltestning gjordes en detaljerad analys av den somatosensoriska funktionen hos patienter med och utan smärta efter ensidig perifer traumatisk nervskada (Studie I) samt hos patienter med en långvarig smärtlindrande effekt på minst 30 % efter ryggmärgsstimulering (Studie IV). Kombinationen av en differentierad nervblockad och upprepad kvantitativ känseltestning användes för att fastställa vilken typ av nervfibrer som bidrar till smärta utlöst av 1 s respektive 10 s stimulering av huden med von Frey filament (Studie II). Samma metodik användes för att kartlägga vilka perifera fibrer som är substratet för dynamisk mekanisk allodyni och dysestesi (Studie III).

Resultat: Patienter med smärta uppvisade allodyni för kyla och tryck tillsammans med en ökad perceptionströskel för icke smärtsam värme på den skadade sidan jämfört med kontrollsidan. Patienter utan smärta hade ökade perceptionströsklar för lätt beröring, kyla och värme på den skadade sidan. Ingen signifikant skillnad kunde påvisas vid jämförelser av sidoskillnader mellan patienter med och utan smärta. Under nervblockaden sågs en minskad känslighet för smärta utlöst av både 1 s respektive 10 s stimulering av huden med von Frey filament. Denna förändring inträffade samtidigt för både kort och längre stimuleringstid och signifikant före en ökning av perceptionströsklarna för både kyla och värme. Under nervblockaden sågs även en övergång från dynamisk mekanisk allodyni till dynamisk mekanisk dysestesi hos alla patienter med perifer neuropatisk smärta och hos 3/7 patienter med central smärta efter en stroke. Övriga patienter förlorade sin dynamiska mekaniska allodyni utan övergång till dysestesi. Både övergång och förlust av dynamisk mekanisk allodyni inträffade tidigt under nervblockaden när i huvudsak endast nervfibrer som förmedlar beröring var påverkade. Efter ryggmärgsstimulering påvisades en sänkt perceptionströskel för lätt beröring och en ökad perceptionströskel för trycksmärta i det neuropatiska området

jämfört med före stimulering. Jämfört med den motsatta sidan förändrades dessa perceptionströsklar mot normalisering och inkluderande även en signifikant normalisering av perceptionströskeln för kyla. Ryggmärgsstimulering förändrade inte känsligheten för smärtsam temperaturstimulering. Ingen signifikant korrelation kunde påvisas mellan grad av tröskelförändring och grad av smärtlindring.

Slutsatser: Fyndet av ökad smärtkänslighet för kyla och tryck på den skadade sidan hos patienter med smärta efter traumatisk perifer nervskada talar för överretbarhet i smärtsystemet, vilket dock inte kunde bekräftas av en mer utmanande statistisk analys av sidoskillnader mellan patienter med och utan smärta. Nervfibrer som normalt förmedlar beröring är det perifera underlaget för smärta utlöst av både 1 s respektive 10 s stimulering av huden med von Frey filament. Dock kan olika receptororgan vara involverade d.v.s. snabbt adapterande respektive långsamt adapterande mekanoreceptorer. Övergången från allodyni till dysestesi och bortfallet av dynamisk mekanisk allodyni utan övergång uppstod tidigt och samtidigt under en differentierad nervblockad parallellt med en kontinuerlig försämring av funktionen hos i huvudsak de nervfibrer som förmedlar beröring. Vi föreslår därför att dynamisk mekanisk allodyni är en förstärkning av dynamisk mekanisk dysestesi där skillnaden i upplevelse beror på antalet mekanoreceptiva fibrer som har kontakt med smärtsystemet. Förändrad sensorisk funktion efter ryggmärgsstimulering indikerar en koppling till bortfall av en funktionell blockering av somatosensorisk funktion framkallad av aktivitet i smärtsystemet. Ingen signifikant korrelation kunde påvisas mellan grad av tröskelförändring och grad av smärtlindring efter ryggmärgsstimulering.

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